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(21) (A1) 2,148,898

(22) 1995/05/08

(43) 1995/11/10

(51) Int.Cl.[6] C12N 15/54; C12N 9/12; C12Q 1/48; C07K 16/40

(19)(CA) APPLICATION FOR CANADIAN PATENT (12)

(54) P54 Stress-Activated Protein Kinases

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(30) (US) 08/240,014 1994/05/09

(57) 32 Claims

p54 STRESS-ACTIVATED PROTEIN KINASES

Abstract of the Disclosure

The p54 Stress-Activated Protein Kinases (SAPKs) are members of a large gene family and are structurally related to the previously described mitogen-activated protein kinases (MAPKs). They are distinguished by their strong activation in response to heat shock, TNF-α, IL-1-β, sphingomyelinases, chemical protein synthesis inhibitors, and ischemia. These kinases have potential utility in the modulation of the inflammatory response and the up-regulation of repair or protective cellular proteins following injury or chemical insult.

124798.B11

PATENT

ATTORNEY DOCKET NO: 00786/217CA1

p54 STRESS-ACTIVATED PROTEIN KINASES

Statement as to Federally Sponsored Research

This invention was funded at least in part by the following grants from the United States Government: GM46577 to JMK, DK17776 and DK41513 to JA (DK41513 transferred to JMK as of 1/94), and the U.S. Government has certain rights in the invention.

Background of the Invention

The field of the invention is second messenger protein kinases which regulate activation of transcription factors, which in turn modulate cellular responses to extracellular stimuli.

The extracellular signal-regulated kinases (ERKs) are a family of proline-directed kinases that are activated via concomitant Tyr and Thr phosphorylation and share sequence homology in the Ser/Thr protein kinase catalytic domain. They include the mitogen activated protein kinases, or MAPKs. Stimuli for the activation of these kinases are diverse, and induce discrete second messenger pathways to effect specific cellular responses. ERKs participate in the regulation of other protein kinases and several transcription factors including c-Jun, c-Myc, c-Fos, ATF-2, and $p62^{TCF}$/Elk-1. These functions indicate that the ERKs mediate the expression of genes in response to extracellular agonists.

The first well characterized members of this kinase family were p42 and p44 MAPKs, which are stimulated by insulin and require both Tyr and Thr phosphorylation for activity (Sturgill *et al*, *Nature* 334:715-718, 1988; Anderson *et al.*, *Nature* 343:651-653, 1990). They are also stimulated by a variety of mitogens, phorbol esters, and activated *ras*.

The ERKs share sequence homology in the Ser/Thr protein kinase catalytic domain, as mentioned above, and this functions to phosphorylate c-Jun on serine and threonine residues that have been localized to c-Jun tryptic peptides termed X and Y. X and Y are located near the N-terminal *trans*-activation domain (Pulverer *et al.*, *Nature* 353:670-673, 1992). Phosphorylation of these peptides regulates transactivating binding activity, and thus their function as promoters of gene expression.

Summary of the Invention

The invention features a molecule consisting of either DNA or amino acids encoding a p54 stress-activated protein kinase (SAPK), or a biologically active fragment thereof, characterized by its ability to modulate transcription pathways in response to extracellular stress stimuli. By "biologically active fragment" it is meant that the fragment can exert a physiological effect (e.g., binding to its biological substrate, phosphorylation, causing an antigenic response, etc.) *in vivo* or *in vitro*.

In preferred embodiments, the molecule of the invention is a polypeptide at least 95% identical to p54αI, p54αII, p54βI, p54βII, or p54γ, preferably from a mammalian source (e.g., SEQ ID NOs: 1, 2, 3, 4, or 5). Any polypeptide sequence containing an "X" is intended to denote a position that could be any amino acid. By "polypeptide", it is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

Other preferred embodiments include polypeptides that are substantially identical to SEQ ID NOs 1, 2, 3, 4, 5, 6, 7, and 8. By "substantially identical", it is meant an amino acid sequence which differs only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same

class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the biological function of the polypeptide.

In one preferred embodiment, the molecule of the invention is a polypeptide fragment that contains a biologically active portion of a p54 polypeptide, at least 10 amino acids in length. Examples include, but are not limited to, the ATP binding site, which includes Lys_{55} and $Gly_{33, 35, and 38}$; the site of regulatory phosphorylation by upstream activators (Thr_{183}-Pro-Tyr_{185}); and the SAPK catalytic domain (between amino acids 22 and 321; see Fig. 1).

In another preferred embodiment the polypeptide or a fragment thereof is useful for producing antibodies which specifically bind to a p54 stress-activated protein kinase (e.g., SEQ ID NOs 6, 7, and 8). In this context, fragment means at least the smallest antigenic epitope, generally at least 10 contiguous amino acids.

The invention also features a DNA molecule encoding a p54 stress-activated protein kinase polypeptide, its degenerate variants, or a fragment thereof including at least 30 contiguous nucleotides. The DNA sequence may be 90% identical to p54αI (e.g., SEQ ID NO: 9), p54αII (e.g., SEQ ID NO: 10), p54βI (e.g., SEQ ID NO: 11), p54βII (e.g., SEQ ID NO: 12), or p54γ (e.g., SEQ ID NO: 13), or be a fragment of any of the above nucleotide sequences containing at least 30 contiguous nucleic acids, or a degenerate variant thereof. By "degenerate variant" it is meant any nucleotide sequence that encodes the same amino acid sequence as the polypeptide translated from the DNA, or a substantially identical polypeptide.

The molecules of the invention are preferably derived from a mammal, more preferably from a rat or human.

The invention also includes DNA molecules which hybridize under stringent conditions to one or more of the DNAs encoding p54 SAPKs (SEQ ID NOs 9, 10, 11, 12, and 13). By "stringent conditions" it is meant conditions under which molecules without significant homology (e.g., at least 90%) to the DNAs of the invention could not hybridize (protocols to determine stringency and melting point of DNA sequences are well known to those skilled in the art and may be found in Sambrook et al. (eds), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, 1989; hereby incorporated by reference). Included in the term "DNA(s)" are the sequences of both strands of double stranded DNA.

The invention also features methods of screening potentially therapeutic compounds involving applying test compounds in physiologically relevant concentrations in a suitable excipient to cultured cells, with or without previous, prior, or concurrent stress-activated protein kinase-activating stimuli (e.g., TNF-α, IL-1-β, sphingomyelinase, heat shock, etc.), preparing cellular extracts, and assaying the isolated recombinant stress-activated protein kinase for c-Jun kinase activity. By "physiologically relevant" it is meant a concentration that is achievable during non-toxic administration to a human patient. By suitable excipient it is meant a non-toxic or non-injurious solvent or carrier for the molecules of the invention or test compounds used in the screening assay.

In another embodiment, the cells treated with the test compound and with or without previous, prior, or concurrent SAPK activating stimuli are extracted, and

combined with inactive recombinant stress-activated protein kinase. The recombinant SAPK is then isolated and assayed for c-Jun kinase activating ability.

The advantages and uses of the invention are in the treatment and prevention of inflammation and the deleterious effects of hypoxia, heat stress, reperfusion injury, and other tissue insults which are currently difficult to manage clinically. The molecules of the invention may be useful for reducing inflammation in such chronic disorders as autoimmune diseases or allergies, and in acute conditions such as anaphylactic shock, or soft tissue injury where swelling may aggravate the condition (e.g, around broken bones). Other uses include prophylactic treatment of patients about to undergo surgeries where there is a high likelihood of ischemia-reperfusion injury (e.g., vascular surgery, organ transplants, etc.), or treatment of sepsis and fever. Additionally, these molecules could be used to up-regulate AP-1 expression via c-Jun phosphorylation, and thus enhance levels of IL-2 expression as a cancer therapeutic.

The molecules of the invention may also be useful for drug design based on an understanding of the enzymes' structure and function (e.g., the polypeptide's binding site on c-Jun), or as indicators in an assay to screen large numbers of drugs for beneficial effects on the conditions listed above.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

Brief Description of the Drawings

Figure 1: Deduced amino acid sequences (SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, and 8) alignment of p54 stress-activated protein kinase (SAPK) cDNAs (single

letter code). Sequences determined by protein microsequencing are underlined, and the black diamonds flank the catalytic domains of the SAPKs.

Figures 2 a) and b): Specificity of anti p54 SAPK antisera for p54 SAPKs. a) *In vitro* translation of ERKs. RNAs (2μg) transcribed from plasmids containing the cDNAs of rat p54αI (lane 1), p54β (lane 2), and HA-epitope-tagged p44 MAPK (lane 3) were translated using rabbit reticulocyte lysates. Lane 4 shows lysate programmed with water; a 43-kDa background band is indicated by an open arrowhead. b) Aliquots of the translated proteins were immunoprecipitated in RIPA buffer with antisera raised against bacterially expressed p54β. Lanes are the same as in the left panel (lanes 1, 2, and 3).

Figures 3 a) and b): HepG2 cells were treated with TNF-α (100ng/ml, 15 min). Cell extracts were prepared and depleted of SAPKs by exhaustive immunoprecipitation for the times indicated (a) or for 4 hrs (b), at which time extracts were subjected to GST-c-Jun chromatography as a means of determining the c-Jun kinase activity remaining in the extracts. As a control, immunodepletions were done with preimmune serum or without serum (b). Activity in the immunoprecipitates and on the c-Jun columns was measure (a). The results indicate that around 70% of the c-Jun kinase activity present in the extracts can be removed with the anti-SAPK serum.

Figures 4 a) and b): Comparative activation of p42/44 MAPKs (open bars) and p54 SAPKs (filled bars) showing that NIH3T3 cell (a) and HT-29 cell (b) p54 SAPKs are poorly activated by mitogens and strongly activated by heat stress and cycloheximide.

Figure 5 a) and b): Activation of c-Jun phosphorylation *in situ* by various stimuli. a) c-Jun phosphorylated *in vivo* in response to cycloheximide (lane

1) and heat shock (lane 2); retardation of the Jun polypeptide upon SDS-PAGE (compare lanes 1 and 2 with non-phosphorylated c-Jun in lane 3). The c-Jun polypeptide is indicated with an arrowhead. b) Two dimensional tryptic phosphopeptide mapping of c-Jun polypeptides immunoprecipitated from control (left), or heat shock-treated (right) HepG2 cells indicates enhanced phosphorylation of peptides X and Y in response to heat shock. The origin is marked with an arrowhead. The dotted circle indicates the position of the xylene cyanol marker.

Figures 6 a) and b): Detection and isolation of c-Jun kinases activated by heat shock and other stimuli on GST-c-Jun immobilized on glutathione agarose (b); comparison with activation of p54 SAPKs (a). The black and white bars each represent results from one experiment.

Figures 7 a) and b): Activation of SAPKs and c-Jun kinases by tunicamycin. p54 SAPK activity is shown in a, closed circles (mean ± SD for triplicate determinations), total c-Jun kinases binding to immobilized GST-c-Jun, a, open circles; b shows the fold activation of the p42/p44 MAPKs by tunicamycin.

Figure 8 a) and b): Comparison of activation of p54 SAPKs and p42/44 MAPKs by various stimuli in human CCD-18Co cells. a) Activation of p54 SAPKs by TNF-α and other stimuli. Mean ± SD for triplicate determinations is shown. b) Parallel assays for relative activation of p42/44 MAPKs by the same stimuli shown in a).

Figure 9 a) and b): Comparison of activation of p54 SAPKs and p42/44 MAPKs by various stimuli in primary porcine hepatocytes. a) Activation of p54 SAPKs by TNF-α and other stimuli. Mean ± SD for triplicate determinations is shown. b) Comparison of fold

activation by various stimuli of p54 SAPKs (filled bars) and p42/44 MAPKs (open bars).

Figure 10: Activation of HepG2 cell p54 SAPKs by TNF-α and *S. aureus* sphingomyelinase. SMase = sphingomyelinase.

Figures 11 a) and b): Comparison of activation of p54 SAPKs and p42/44 MAPKs by IL-1-β. EL-4 murine thyoma cells were treated with 20 ng/ml recombinant human IL-1-β. Cells were extracted as described in the legend of Table 2. SAPKs (a and filled bars, b) and p42/44 MAPKs (b) were assayed in standard experimental paradigms. For a) data are mean ± SD for triplicate determinations. For b) data are presented as percent of control for comparative purposes.

Figures 12 a) and b): Effect of ischemia/reperfusion on MAPK and SAPK activation *in vivo*. Activation of p54 SAPK (a) and p42/44 MAPKs (b) was measured at times from 0-90 min after initiating reperfusion of rat kidneys made ischemic for 45 min. as described in the Methods section. MAPKs experienced rapid activation and inactivation (b), while SAPKs were rapidly activated and remained at elevated levels for periods well in excess of 90 min.

Figure 13: Bacterially expressed rat SAPK-α was expressed as a GST fusion protein and purified on glutathione agarose. The SAPK was treated with buffer alone, or with an extract from HepG2 cells treated with TNF and prepared as described in the Table 2 legend. The SAPK was recovered with glutathione beads and assayed for c-Jun kinase activity. As an additional control, blank beads were exposed to cell extracts (extract alone bar).

Description of the Preferred Embodiments

Methods

Cloning

p54 SAP kinase was purified to homogeneity from livers of cycloheximide injected rats (Kyriakis and Avruch, *J. Biol. Chem.* 265:17355-17363) and the sequences of several tryptic peptides were determined following RP-HPLC. Two of these peptides (HRDLKPSN and MLVIDPDKRISVDEAL) were homologous to protein kinase subdomains VIb and XI, respectively (Hanks *et al.*, *Science*, 241:42-52, 1988) and were used to design degenerate sense and antisense primers, respectively. A 467 bp fragment was amplified by PCR from rat brain cDNA and used as a probe to screen 250,000 plaques of a rat brain cDNA library in λZAP (Stratagene). Twenty seven positive plaques were purified and representatives sequenced on both strands using nested deletion series.

Antisera/Antibodies

To generate antisera, the p54 SAPK-β isoform was subcloned into pGEX-KG and expressed as a glutathione S-transferase (GST) fusion protein. Expression p54 SAPK-β polypeptide was induced with IPTG (50μg/ml) and the fusion purified to homogeneity by glutathione-agarose chromatography, followed by thrombin cleavage to release the kinase moiety from the fusion protein. This material was then used as an immunogen and antisera collected and tested. The antisera tested have been shown to cross-react with human, mouse, rat, and pig tissues. The SAPK sera react with several human cell lines, including human hepatocellular carcinoma cells (HepG2), human colon fibroblasts (CCD-18Co), human monocytic lymphoma cells (U937), and human colon carcinoma cells (HT-29).

Monoclonal antibodies may be made by fusing immune B cells from the spleen with tumor cells to produce

hybridomas specifically secreting each antibody, using methods well known in the art (see, for instance, Coligan *et al.*, eds. Current Protocols in Immunology, John Wiley and Sons, 1992; Kohler *et al.*, *Nature* 256:495, 1975; Hammerling *et al.*, in Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981)

Peptide antisera were generated using standard methods to the p54α, p54β, and p54γ classes using the least conserved region of the molecules (SEQ ID NOs: 6, 7, and 8) to enhance the probability of class-specificity.

Cell Culture Treatments and Assay

Confluent cultures of NIH3T3 cells were treated with H_2O_2 (5 mM, 15 min) phorbol-12-myristate-13-acetate (PMA, 500 nM, 20 min) FGF (10 ng/ml, 20 min), A23187 (100 nM 20 min), heat (42°C, 30 min) or cycloheximide. HT-29 cells were treated with EGF (50 ng/ml, 20 min) or heat shock (42°C, 30 min). Cells were washed three times in PBS and lysed in ice-cold lysis buffer (20 mM Na-Hepes, pH 7.5, 2 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4. 50 mM β-glycerophosphate, 1% (w/v) Triton X-100, 10% (v/v) glycerol, 2 μM leupeptin, 10 kallikrein-inhibiting units/ml aprotinin, 200 μm PMSF). Extracts were normalized to identical protein concentration (1 mg/ml). A portion (1 ml) was immunoprecipitated with 5/13-99, and assayed for GST-Jun kinase activity as follows. To 40 μl of a 1:1 suspension of p54 SAPK beads were added 20 μl 0.2 mg/ml GST-c-Jun-1-135 (Adler *et al.*, (1992), *Proc. Natl. Acad. Sci. U.S.A.*, 89:5341-5345) or 0.01 mg/ml holo-c-Jun (Pulverer *et al.*, *supra*; Pulverer *et al.*, (1993), *Oncogene*, 8:407-415). ATP (100 μM) and $MgCl_2$ (10 mM) were added to start the reaction. The reaction was allowed to proceed for 20 min at 30°C at which time the reaction was stopped with SDS sample buffer and the

mixtures resolved by SDS-PAGE. The 40-kDa GST-Jun band was excised and counted by liquid scintillation spectroscopy (mean ± SD for triplicate determinations are shown in the figures). Another portion (1 ml) was assayed for p42/p44 MAPK activity by Mono-Q chromatography, and by a myelin basic protein (MBP) kinase activity assay (Ahn *et al.*, (1991), *J. Biol. Chem.*, 266:4220-4227). A peak of stimulated MBP kinase activity was always detected eluting between 200 and 350 nM NaCl. Total p42/p44 MAPK activity was taken as that contained in those fractions and is shown as percent of control for comparative purposes.

U937 cells were labeled with ^{32}P orthophosphate (1 mCi/ml) and treated with cycloheximide heat stress or vehicle. c-Jun was immunoprecipitated using a polyclonal antibody specific for the C-terminal 15 amino acids of c-Jun and subjected to SDS-PAGE (Pulverer *et al.*). HepG2 cells were labeled with ^{32}P as above and subjected to heat shock. c-Jun was immunoprecipitated and, after SDS-PAGE, was subjected to two dimensional tryptic phosphopeptide mapping. In other experiments, U937 cells were treated with actinomycin-D (10 µg/ml, 30 min), anisomycin (10 µg/ml, 30 min) emetine (10 µg/ml, 30 min), puromycin (10 µg/ml, 30 min) or heat stress (42°C, 30 min). Lysates were prepared and were passed over columns of immobilized GST-Jun. After washing away unbound proteins, Mg/$\gamma^{32}P$-ATP was added and phosphorylation of the immobilized c-Jun was assayed as described above. Parallel aliquots were subjected to immunoprecipitation and assayed for p54 SAPK. In both assays, 1 U of kinase activity transferred 1 pmol PO_4/min from ATP to GST-Jun. HT29 cells were treated with various concentrations of tunicamycin for 5 h. Lysis, assay of p42/p44 MAPK activity, and p54 SAPK immune complex kinase assay were done by standard methods. Detection of total tunicamycin-stimulated c-Jun

kinase activity in HT-29 cells isolated on columns of immobilized c-Jun was performed as described above.

CCD-18Co cells, HepG2 cells, or primary porcine hepatocytes were cultured to 80% confluence, serum starved (0.5% serum, 16 hours) and treated with the following agonists as shown in Figures 16 and 17: heat shock (42°C, 30 min), PMA (200 nM, 20 min), EGF (100 ng/ml, 20 min) or TNF-α (50 ng/ml, 10 or 20 min). Extracts were prepared and p54 SAPKs were immunoprecipitated and assayed. Parallel assays of p42/p44 MAPKs were performed. For treatment with sphingomyelinase, 100 mU/ml *S. aureus* sphingomyelinase were added to HepG2 cells as known in the art (Dressler *et al.*, (1992), *Science*, 255:1715-1718) for 15 min. 1U *S. aureus* sphingomyelinase hydrolyzed 1 μmole TNP-sphingomyelin/min at pH 7.4, 37°C.

Ischemia/Reperfusion

Male Sprague-Dawley rats, 120-150 g each, were anesthetized with sodium pentobarbital (65 mg/kg). In order to induce ischemia, the renal artery of one kidney was clamped for 45 min. The contralateral kidney served as a control. Reperfusion was accomplished by releasing the clamp and allowing blood flow for 0 to 90 min. Control and ischemia/reperfusion kidneys were harvested and homogenized in lysis buffer (20 mM Hepes, pH 7.4, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM DTT, 250 mM sucrose, 400 μM PMSF, 2 μM leupeptin, 2 μM aprotinin). After centrifugation for 1 hr at 100,000 x g, the supernatants were collected and Na_3VO_4 added to 0.1% (w/v). Immunoprecipitation and SAPK assay were as described in the Table 2 legend.

Isolated clones

p54 protein kinase was isolated from rat liver that had been stimulated with cycloheximide, using the above methods (also described in Kyriakis and Avruch, *supra*; hereby incorporated by reference) and amino acid sequences were derived from peptides generated by tryptic digests. These sequences aligned with the consensus Ser/Thr protein kinase catalytic domain of known MAPKs (Hanks *et al.*). Two of these peptide sequences were used to design overlapping degenerate oligonucleotide probes for use in a nested PCR reaction from which a 467 bp cDNA was generated using rat brain cDNA as the template. This probe was used to screen a rat brain cDNA library from which 27 clones were isolated and sequenced. The cDNAs encoded five separate polypeptides (Fig. 1, SEQ ID NOs 1-5). This was quite unexpected, since it was assumed that only one peptide had been purified to homogeneity from the rat liver in this and previous work (Kyriakis and Avruch). One set of clones (p54αI, SEQ ID NO. 9) encoded a protein that contained all of the tryptic peptides derived from rat liver p54 kinase. Two additional sets (p54βI/II, SEQ ID NOs 11 and 12; and p54γ, SEQ ID NO. 13) encoded closely related polypeptides (88-90% identity, respectively to αI, Fig. 1). A fourth group of cDNAs (p54αII, SEQ ID NO. 10) was identical to αI kinase except for a region of 71 base pairs which results in the substitution of 15 amino acids in subdomains IX and X (see Fig. 1). The αI and αII RNAs are most likely derived from the same gene by alternate splicing. The predicted proteins encoded by the full length clones are: αI, 48,076 Da; αII, 47,986 Da; and βI, 48,095 Da. Northern blotting analysis of several tissues revealed ubiquitous, low expression of all three gene classes.

Properties of p54 SAPKs

The distinct characteristics of the isolated p54 clones and expressed and native polypeptides have led to the nomenclature of Stress-Activated Protein Kinases (SAPKs), which will be used throughout to distinguish them from the MAPKs.

Sequence alignment of the catalytic domains of the p54 SAPK clones with those of the known mammalian MAPKs, and with the yeast MAPK homologs *KSS1*, *HOG-1* *FUS3*, *SLT-2*, *spk-1* and *erk-1* (Courchesne *et al.*, (1989), *Cell*, 58:1107-1119; Brewster *et al.*, (1993), *Science*, 259:1760-1763; Levin *et al.*, (1993), *J. NIH Res.*, 5:49-52) shows that the p54 polypeptides exhibit nearly equal identity to the mammalian 44 kDa MAPK (43-44% sequence identity) and the kinases from lower eukaryotes (41-44% identity). By contrast, p44 MAPK is closer in sequence to the yeast kinases (between 49-52% for the *S. cerevisiae* enzymes and 56% identity for Spk-1) than it is to the p54 SAPKs. From these and other comparative data, we conclude that none of the MAP kinase-like genes identified thus far in lower eukaryotes is likely to be a functional homologue of the p54 SAPKs. All of the p54 isoforms contain the sequence Thr_{183}-Pro-Tyr_{185} in an analogous position to the Thr and Tyr residues of p42/p44 MAPKs (Payne *et al.*, (1991) *EMBO J.* 10:885-892) that must be phosphorylated for activity. These residues in the p42/p44 MAPKs are phosphorylated by the MAPK or ERK Kinases (MEKs), a family of dual specificity protein Tyr/Thr kinases (Ahn *et al.*; Gómez *et al.*, (1991) *Nature*, 253:170-173; Nakielny *et al.*, (1992), *EMBO J.*, 11:2123-2129). However, *in vitro*, neither purified dephosphorylated liver p54 SAPK nor bacterially expressed p54 SAPK isoforms are phosphorylated or reactivated by p42/p44 MAPK-specific MEKs, suggesting the existence of a specific p54 SAPK kinase.

In vivo administration of cycloheximide activates p54 SAPK, and we have also characterized the stimuli and signal transduction pathways that activate p54 SAPKs in cultured cells. A polyclonal antiserum (5/13-99), raised against the prokaryotic recombinant p54 SAPK β-isoform, immunoblots p54 SAPK and immunoprecipitates *in vitro* translated p54α and p54β polypeptides but not p44 MAP kinase (Fig. 2). Purified rat liver p54 SAPK phosphorylates c-Jun exclusively at Ser_{73} and Ser_{63} in the c-Jun *trans* activation domain, two sites located on c-Jun tryptic phosphopeptides designated X and Y, respectively (Pulverer *et al.*; Smeal *et al.*, (1991), *Nature*, 354:494-496). The anti-p54 SAPK antiserum (5/13-99) immunoprecipitates from HT-29 human colon carcinoma cells a protein kinase activity that phosphorylates recombinant GST-c-Jun, as well as full-length c-Jun, selectively at tryptic peptides X and Y. Pretreatment of NIH3T3 cells or HT-29 cells with cycloheximide substantially augments this c-Jun (X/Y) kinase activity. Thus, the p54 antiserum is reactive with p54 SAPKs but not p42 MAPK or p44 MAPK. Moreover, p54 SAPK activity, measured as a c-Jun "X/Y" kinase, is stimulated by cycloheximide in tissue culture as well as *in vivo*.

SAPKs are the major c-Jun kinase activated by TNF-α. SAPK immunodepletion experiments removed 60-70% of the recombinant c-Jun kinase activity induced in TNF-α treated cells (Fig. 3). This indicates that the SAPKs account for around 70% of the c-Jun-associated c-Jun kinase activity.

Regulation of p54 SAPKs

Effects of MAPK Activators

We examined the regulation of the p54 SAPK, in NIH3T3 and human HT-29 cells, by agents known to regulate p42/p44 MAPKs. The activity of p54 SAPKs was measured in

an immune complex kinase assay using GST-c-Jun as a substrate, whereas the p42/p44 MAPKs in the same extracts were assayed, after Mono-Q anion exchange chromatography, using MBP as a substrate. Figure 4 compares the activation of each set of kinases in response to the various treatments to the baseline values. As expected, NIH3T3 cell p42/44 MAPKs are strongly activated by mitogen (10-fold activation by FGF) and phorbol esters (6-fold), and are activated to a lesser extent by Ca^{2+} influx stimulated by the ionophore A23187 (2-fold). In addition, the p42/p44 MAPKs are activated by H_2O_2 and cycloheximide (4- and 6-fold, respectively). In striking contrast, the NIH3T3 cell p54 SAPKs are not activated by FGF, phorbol esters, H_2O_2 or by Ca^{2+} ionophore (top panel, filled bars). A similar result is seen for HT-29 cells (bottom panel, filled bars). In these cells, EGF substantially stimulates the p42/p44 MAPKs (6 fold, bottom panel, open bars) while only slightly stimulating p54 SAPKs (bottom panel, filled bars).

These results suggest that signals generated by activation of receptor tyrosine kinases and phospholipase C are not likely to represent the primary regulatory input to the p54 SAPKs. Therefore, stimuli other than mitogens were investigated to see if they could activate p54 SAPKs more vigorously than they activated the p42/p44 MAPKs. Cycloheximide, although capable of strongly activating the NIH3T3 cell p54 SAPK (10-fold), also gives substantial (5-fold) activation of endogenous p42/p44 MAPKs. By contrast, heat shock (42°C, 30 min) gives a large induction of NIH3T3 cell p54 SAPK activity (7-fold), while p42/p44 MAPK activity is only slightly (1.5-fold) stimulated (Fig. 4, top panel). In HT-29 cells as well, heat shock dramatically stimulates HT-29 cell p54 SAPKs (8.4 fold, Fig. 4, bottom panel, filled bars) while only modestly stimulating p42/p44 MAPKs (2.9 fold, Fig.

4, bottom, open bars). Activation of p54 SAPK by heat shock (42°C) is evident by 10 min, maximal at 30 min, and declines slightly after 1 hr.

Effect of p54 SAPK Activation on c-Jun

Since the p54 SAPKs are potent c-Jun kinases *in vitro*, we inquired whether stimuli such as heat shock and cycloheximide, that activate p54 SAPKs preferentially, also increase the phosphorylation of c-Jun *in situ*. ^{32}P-labeled U937 and HepG2 cells were exposed to heat shock or cycloheximide, and the phosphorylation of endogenous c-Jun was examined. Both treatments induce a robust phosphorylation of c-Jun, accompanied by a dramatic retardation of c-Jun mobility on SDS-PAGE (Fig. 5a). This retardation is characteristic of phosphorylation on tryptic peptides X and Y (corresponding to Ser 73 and 63, respectively) within the c-Jun N-terminal *trans* activation domain (Pulverer *et al.*); the induction of X/Y phosphorylation was verified directly by tryptic peptide maps of ^{32}P-c-Jun isolated from heat-shocked ^{32}P-labeled HepG2 cells (Fig. 5b). Activation of c-Jun N-terminal phosphorylation by heat shock represents a previously unrecognized mode of c-Jun regulation. The likelihood that p54 SAPKs contribute substantially to the c-Jun phosphorylation elicited by heat shock and cycloheximide *in situ* is supported by the ability of the immunoprecipitated p54 SAPK activated by cycloheximide to phosphorylate recombinant c-Jun *in vitro* exclusively on tryptic ^{32}P peptides that co-migrate with tryptic peptides X and Y (see Fig. 3).

Effects of Protein Synthesis Inhibitors on p54 SAPKs

Maneuvers that increase c-Jun X/Y phosphorylation *in situ*, such as UV light or PMA, have been shown to activate c-Jun kinases that bind tightly to immobilized

GST-Jun (Adler *et al.*; Hibi *et al.*, *Genes & Dev.*, 7:2135-2148, 1993). Extracts from heat-shocked or cycloheximide-treated cells also contain activated c-Jun kinase(s) that bind to immobilized GST-c-Jun (Fig. 6, right panel). Moreover, two other inhibitors of polypeptide chain elongation, emetine and especially anisomycin, also activate both p54 SAPK and GST-Jun-bound c-Jun kinase (Fig. 6), whereas puromycin and the RNA synthesis inhibitor, actinomycin D, are each unable to activate either c-Jun kinase (Fig. 6, right panel) or p54 SAPK activity (Fig. 6, left panel). A summary of these results is presented in Table 1, below. Thus, heat shock, a variety of protein synthesis inhibitors as well as the glycosylation inhibitor, tunicamycin (see below), alter p54 SAPK, and the GST-Jun-bound c-Jun kinase in parallel, suggesting that p54 SAPK is likely to be one of the physiologic Jun kinases activated by this class of perturbations.

TABLE 1

Activation of p54s and c-Jun-associated kinases by protein and RNA synthesis inhibitors.

Treatment	p54 MAPK (mU)	c-Jun-associated kinase (mU)
Control	31.0	14.5
Anisomycin	370.0	385.0
Cycloheximide	166.0	243.0
Heat-shock	168.0	180.0
Emetine	75.0	85.0
Puromycin	19.3	21.5
Actinomycin-D	22.0	24.0

Activation of p54s and c-Jun-associated kinases by protein and RNA synthesis inhibitors. U937 cells were treated with anisomycin (10μg/ml, 30 min), cycloheximide

(200μM, 60 min), heat shock (42°C, 30 min), or actinomycin D (10μg/ml, 30 min). Extracts were prepared as described in the methods, and parallel aliquots were subjected to GST-c-Jun chromatography/assay or immunoprecipitation and assay for p54. In both assays, 1 unit of kinase activity transferred 1 pmol PO_4/min from ATP to GST-c-Jun. Shown are mean results for two experiments.

Polypeptide Misfolding Stimulates p54 SAPKs

Although heat shock and protein synthesis inhibitors could activate p54 SAPK through entirely unrelated mechanisms, a shared property of heat shock and translational inhibitors (but not the RNA synthesis inhibitor, actinomycin D), is their ability to promote polypeptide misfolding and denaturation. Consequently, we investigated whether the accumulation of misfolded polypeptides might be an initial stimulus common to protein synthesis inhibitors and heat shock that leads to the preferential activation of p54 SAPK. Tunicamycin inhibits the synthesis and proper folding of proteins destined for membrane insertion or secretion through inhibition of N-linked glycosylation in the Golgi. Increasing doses of tunicamycin added to HT29 cells promotes a striking activation (up to 12-fold) of p54 SAPK activity (Fig. 7, top, filled circles), whereas the p42/p44 MAPKs, assayed after separation by Mono-Q chromatography, are only modestly activated (Fig. 7, bottom). In addition, tunicamycin stimulates total HT-29 Jun kinases which bind to GST-c-Jun with a similar dose response to p54 SAPK activation (Fig. 7, top, open circles). The activation of the p54 SAPKs by tunicamycin, together with the data in Figures 4 and 6 support the idea that cellular stresses which result in the accumulation of misfolded polypeptides, can, to a degree greater than mitogens, generate a signal to activate the p54 SAPKs. By contrast, the p42/p44 MAPKs

are more strongly activated by mitogenic signaling, relative to stress signaling.

Cytokines TNF-α and IL-1-β

In mammals, integration of the multicellular and inter-organ response to a variety of "stressful" noxious stimuli is mediated by a diverse array of inflammatory cytokines, such as TNF-α and IL-1-β. TNF-α was initially detected by its ability to induce the hemorrhagic necrosis of some transplantable tumors in inbred mice (Buetler *et al.*, *Ann. Rev. Biochem.*, 57:505-517, 1988; Goeddel *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51:597-609, 1986). The cellular responses to inflammatory cytokines are quite diverse and cell-specific, and are directed at optimizing overall host defense against infection. For example, TNF-α acts on adipose tissue to inhibit insulin action and energy storage; on liver to yield the protein secretory pattern known as the acute phase response; on neutrophils to enhance superoxide radical production and cytotoxic efficacy; and on T cells to promote the secretion of additional cytokines, e.g. IL-2 and IL-6 (see Buetler *et al.* and Goeddel *et al.* for review). The multifarious actions of TNF-α are due in part to TNF-α-directed programs of gene expression. Notably, TNF-α has been shown to be a potent activator of the *trans* activation function and autoinduction of c-Jun (Brenner *et al.*, *Nature*, 337:661-663, 1989), as well as an activator of NF-κB (Osborn *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 86:2336-2340, 1989), both of which are needed by lymphocytes for *trans* activation of the IL-2 gene.

The profile of physiologic and cellular responses to TNF-α and IL-1-β led us to inquire whether these agents could activate p54 SAPK. Human CCD-18Co colon fibroblasts are acutely responsive to TNF-α (Goeddel *et*

al.); in these cells, TNF-α elicited a striking activation of p54 SAPKs, whereas EGF and PMA were without noteworthy effect (Fig. 8a). The 10-fold stimulation of p54 SAPK by TNF-α was slightly greater than that provoked by heat stress. Comparing the responses of p42/p44 MAPKs in CCD-18Co cells, it is clear that heat stress and TNF-α are far more potent activators of p54 SAPK than are EGF and PMA, while the converse is true for the p42/44 MAPKs (Fig. 8b).

Liver is also a target tissue for TNF-α (Buetler *et al.*, and Goeddel *et al.*); addition of TNF-α to primary cultures of freshly isolated porcine hepatocytes stimulates p54 SAPK activity by ≈ 6-fold (Fig. 9a), whereas EGF increases p54 SAPK activity 3-fold. In these cells, EGF and PMA activate the p42/p44 MAPKs and the p54 SAPKs to a comparable degree. However, as is seen in CCD-18Co cells, heat shock and TNF-α are much more potent activators of p54 SAPK than they are p42/p44 MAPK activators (Fig. 9b). The present results are thus consistent with recent reports showing that TNF-α activates p42/p44 MAPKs (Van Lint *et al.*, *J. Biol. Chem.*, 267:25916-25921, 1992); however, it is clear that the p42/p44 MAP kinases are much more potently activated by ligands like EGF and FGF, that operate through receptor tyrosine kinase, whereas the ability of TNF-α to activate the p54 SAPKs greatly exceeds the ability of FGF and EGF to activate the p54 SAPK, at least in most cell types.

Little is known of the molecular mechanisms of TNF-α signaling. TNF-α binds to one of two receptors, 55-kDa and 70-kDa, whose intracellular extensions show no homology with receptors whose signal transduction mechanisms are better understood (Loetscher *et al.*, *Cell*, 61:351-359, 1990; Heller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 87:6151-6155, 1990). TNF-α has been shown to stimulate rapid sphingomyelin hydrolysis and the

accumulation of ceramide, through the activation of a neutral sphingomyelinase (Dressler *et al.*; Yang *et al.*, *J. Biol. Chem.*, 268:20520-20523, 1993; Dobrowsky *et al.*, *J. Biol. Chem.*, 267:5048-5051, 1992; Dbaibo *et al.*, *J. Biol. Chem.*, 268:17762-17766, 1993; Schütze *et al.*, *Cell*, 71:42-52, 1992). Ceramide has been proposed to serve as a second messenger for TNF-α, analogous to the role envisioned for diacylglycerol in the action of hormones that activate phosphatidylinositol-specific phospholipase C enzymes (Dressler, *et al.*; Yang, Z., *et al.*; Dobrowsky, *et al.*; Dbaibo, *et al.*; Schütze *et al.*; and Schütze, *et al.*). Many of the responses to TNF-α, including growth inhibition, apoptosis, activation of heterotrimeric forms of protein phosphatase-2A, activation of a membrane bound Ser/Thr kinase and, possibly, activation of NF-κB can be elicited by the addition of various ceramide derivatives or bacterial sphingomyelinase to intact or permeabilized cells (Dressler *et al.*; Yang, *et al.*; Dobrowsky, *et al.*; Dbaibo, *et al.*; and Schütze *et al.*). Based on these considerations, we compared the ability of TNF-α, IL-1-β and S. *aureus* sphingomyelinase treatment of HepG2 or EL-4 cells to alter p54 SAPK activity. TNF-α stimulates a robust activation of the p54 SAPKs (15-fold) in these cells, and a substantial activation of p54 SAPKs (5.3-fold) is also evident in response to sphingomyelinase (Fig. 10). These results are consistent with the possibility that sphingomyelin hydrolysis, as is known to occur in response to inflammatory cytokines, may be an early step in the p54 SAPK signal transduction pathway.

When EL-4 murine thyoma cells were treated with 20 ng/ml recombinant human IL-1-β, p54 SAPK activity increased over 4-fold, while p42/44 MAPK activity remained unchanged by the treatment (Fig. 11 a and b). This strong induction of SAPK activity without

concomitant activation of MAPKs mirrors the results with cytokine TNF-α, and indicates a similar inflammation-mediated pathway.

Ischemia/Reperfusion Induces p54 SAPKs

An additional distinction for the p54 SAPKs from the p42/44 MAPKs is the time course of activation in response to reperfusion of ischemic tissue. Rat kidney was made ischemic *in vivo* by the methods described above. The results of this experiment (Fig. 12) showed that the p54 SAPKs and the p42/44 MAPKs both were activated immediately upon reperfusion of the kidney, and that the activation of p54 SAPKs peaked after about 20 min (Fig. 12a), while the p42/44 MAPKs peaked at about 5 min after initiation of reperfusion (Fig. 12b). The duration of p54 SAPK activation was also considerably longer, lasting considerably longer than 90 min before returning to control levels, whereas p42/44 MAPK activation returned to control levels within 20 min.

These findings have important implications in the pathogenesis of chronic problems such as acute renal failure, infarction arising from ischemic heart disease, and surgical induction of ischemia/reperfusion injury. The p54 SAPKs clearly play a more significant role in tissue stresses such as ischemia than do the p42/44 MAPKs, and their effects are more sustained. These results are the first to delineate a signal transduction cascade specifically activated during reperfusion of ischemic tissue. It is known that c-*fos* and some heat shock protein genes are transcribed during reperfusion, and that ischemia results in the generation of oxygen radicals and can lead to tissue damage. The data presented here indicate that SAPKs are activated during reperfusion, and SAPKs target c-Jun. Tissue repair genes, such as the collagenase gene, are regulated by AP-

1, and thus, SAPK activation may represent the initiation of repair processes at the molecular level.

Therapeutic Drug Screening with p54 SAPKs

The experimental approaches described above can be used to screen compounds to identify those with therapeutic potential. Two examples of possible screening protocols follow. These examples are not intended to be limiting.

Example 1

Human (or other mammalian) cell lines such as HepG2, CCD-18Co, U937, and HT-29 can be treated with any test compound, and treated with SAPK activators (e.g., TNF-α, IL-1-β, ATP-depletion/refeeding analogous to ischemia/reperfusion, etc.). The order of treatment can be test compound, then SAPK-activator; SAPK activator then test compound, or both treatments simultaneously. Treatment times can vary from 1 min to several hours, depending on the time necessary to induce changes in the SAPK pathway.

The cells are then extracted, and the endogenous SAPKs are immunoprecipitated as described above. The SAPKs are then tested in a standard c-Jun kinase assay for activity. This method of assaying for SAPK activation can also be employed with tissue samples obtained from patient biopsy, and has been used successfully to detect the activity of SAPKs from samples of rat kidney. In this way, therapeutics can be evaluated for their ability to activate or inhibit the SAPK pathway.

Example 2

Any of the human or rat SAPK clones may be used in an expression vector for recombinant bacterial (or viral, etc.) expression of inactive forms of the SAPK. Because of the high homology between rat and human SAPKs, either

may be used in the assay for therapeutic compounds. Human cell lines such as HepG2, CCD-18Co, U937, and HT-29 can be treated with any compound to evaluate its toxicity and efficacy as a therapeutic in modulating the SAPK/c-Jun kinase pathway.

Cells are treated with the test compound either alone or with a SAPK activating compound (such as TNF-α, etc.; the activator may be applied before or concurrently with the application of the test compound), and then extracted to produce a cytoplasmic lysate. This extract is then combined with the recombinantly expressed, inactive SAPK. The SAPK can be expressed as a GST fusion protein so that it is easily purified on glutathione agarose. After a suitable length of time (minutes to hours) for interaction between the cell lysate and the recombinant inactive SAPK, the recombinant SAPK is isolated by binding to glutathione beads (leaving behind endogenous SAPKs) or by immunoprecipitation with an anti-glutathione antibody (commercially available; any other isolation technique may be used also), and assayed for c-Jun kinase activity. If there is no c-Jun kinase activity, and the test compound was applied to the cells alone, then it may be concluded that this compound does not activate SAPKs. If the test compound was applied with a SAPK activator, then it may be concluded that the test compound inhibits the activation of the SAPK pathway. If c-Jun kinase activity is detected, and the test compound was applied to the cells alone, then the compound activates the SAPK pathway. If the test compound was applied with a SAPK activator, and c-Jun kinase activity is detected, then it may be concluded that a) the compound does not inhibit the SAPK pathway, b) the compound may enhance SAPK activity (which can be quantitatively evaluated), or c) the drug has no effect.

Both of these screening systems allow rapid and extensive testing of many compounds, regardless of their preconceived potential therapeutic use, and may facilitate identification of therapeutics which accelerate tissue repair (increased SAPK pathway activation), or prevent or alleviate allergy, excess swelling, anaphylaxis, etc. (decreased SAPK pathway activation). An example of this assay is shown in Figure 13, using TNF-α as the test compound, and showing controls with the expressed SAPK alone, and the HepG2 cell extract alone to demonstrate that the recombinant form is inactive as expressed, and that the cell extract has no intrinsic activity in the assay.

Summary of Results

This invention identifies the molecular structures of a new subfamily of proline-directed protein kinases whose upstream activators include heat stress, protein synthesis inhibitors, ischemia/reperfusion injury, and the inflammatory cytokines, TNF-α and IL-1-β. This array of regulatory inputs suggests that this kinase subfamily may serve to monitor and form part of the cellular response to a variety of intracellular and extracellular stress signals. The p42/p44 MAPKs, although capable of being activated by these stress treatments, are to a much greater degree activated by mitogens (see Table 2 for summary of results). The mitogenic agents such as EGF, FGF and PMA, which have been shown to activate the p42/p44 MAPKs through a Ras and c-Raf-1-dependent pathway, activate the SAPKs weakly if at all. This suggests that the SAPKs, although requiring both Ser/Thr and Tyr phosphorylation for activity (Kyriakis *et al.*, (1991), *J. Biol. Chem.*, 266:10043-10046) as do the p42/p44 MAPKs (Anderson *et al.*), lie on an entirely distinct signal transduction pathway. Consistent with

this conclusion is our inability, using highly purified MEK, to reactivate phosphatase-2A inactivated rat liver SAPK, or to activate prokaryotic recombinant SAPK-β under conditions that provide near complete MEK activation of comparable preparations of purified or recombinant p42/p44 MAPK. The SAPKs are thus the proline-directed kinase elements in what is likely to be a new protein kinase cascade, the second such pathway uncovered in mammalian cells. Three independently regulated protein kinase cascades upstream of distinct proline-directed kinases (*FUS/KSS; HOG-1; MPK-1*) have been uncovered thus far in *S. cerevisiae* (Levin *et al.*). We propose that the SAPK cascade is an important, perhaps central, component of the cellular response system to TNF-α, analogous to the role of the MAPK cascade in the response to activation of receptor tyrosine kinases. Lipid regulators derived from sphingomyelin hydrolysis may participate at one of the earlier intracellular steps upstream of the SAPKs; a similar role for phospholipid-derived mediators upstream of the MAPKs has recently been proposed (Cai *et al.*, (1993), *Mol. Cell. Biol.*, 13:7645-7651).

TABLE 2

Activation of p54 and MAPKs P42/44 In various cell lines by various treatments. ND = not done.

Cells Activity	Treatment	p54 Activity (mU)	p42/44 MAPK
NIH3T3	Control	5.8 ± 0.6	71.4
	PMA	5.8 ± 0.9	409.7
	FGF	8.5 ± 0.6	795.5
	A23187	8.9 ± 0.7	172.0
	H_2O_2	5.9 ± 0.1	272.2
	Heat shock	38.8 ± 1.7	169.7
	Cycloheximide	57.1 ± 4.1	419.9
HT-29	Control	20.7 ± 1.7	95.0
	EGF	35.7 ± 1.4	576.0
	Heat shock	172.0 ± 28.5	281.0
HT-29	Control	69.4 ± 7.5	195.5
	Tunicamycin	803.9 ± 77.1	480.9
CCD-18Co	Control	7.0 ± 1.0	12.2
	PMA	8.8 ± 0.7	601.0
	EGF	13.0 ± 2.1	394.0
	Heat shock	48.0 ± 1.3	83.7
	TNF-α	60.0 ± 5.0	117.6
Primary Porcine Hepatocytes	Control	179 ± 13	1294
	PMA	479 ± 58	3529
	EGF	665 ± 58	4882
	Heat shock	697 ± 49	2427
	TNF-α, 10 min	1008 ± 31	3064
	TNF-α, 20 min	924 ± 38	ND
HepG2	Control	29.8 ± 4.3	ND
	TNF-α	434.8 ± 36.4	ND
	Sphingomyelinase	159.0 ± 17.3	ND

Confluent cultures of NIH3T3 cells were treated with H_2O_2 (5 mM, 15 min) phorbol-12-myristate-13-acetate (PMA, 500 nM, 20 min) FGF (10 ng ml^{-1}, 20 min), A23187 (100 nM 20 min), heat (42°C, 30 min) or cycloheximide (200 μM, 60 min). HT-29 cells were treated with EGF (50 ng ml^{-1}, 20 min), heat shock (42°C, 30 min) or tunicamycin (50 μ ml^{-1}). CCD-18Co Cells (100% confluent) were treated with PMA 200 nM, 15 min), EGF (50 ng ml^{-1}, 15 min), heat shock (42°C, 40 min) or TNF-α (50 ng ml^{-1}, 20 min). Primary porcine hepatocytes were treated the same as the CCD-18Co cells. HepG2 cells (80% confluent) were treated with TNF-α (50 ng ml^{-1}, 15 min) or

S. aureus sphingomyelinase (100 U ml^{-1}). 1U *S. aureus* sphingomyelinase will hydrolyze 1 μmole TNP-sphingomyelin min^{-1} at pH 7.4, 37°C. Cell lysis and immunoprecipitation were performed as described in Kyriakis *et al, Nature* 358:417-421, 1992 (hereby incorporated by reference). Precipitates were assayed for GST-Jun kinase activity as follows. To 40 μl of a 1:1 suspension of protein G Sepharose beads containing immunocomplexed p54 were added 20 μl 0.2 mg ml^{-1} GST-C-Jun-1-135 (26,37) or 0.01 mg ml^{-1} holo-c-Jun. [γ-^{32}P]ATP (100 μM) and $MgCl_2$ (10 mM) were added to start the reaction. The reaction was allowed to proceed for 20 min at 30°C at which time the reaction was stopped with SDS sample buffer and the mixtures resolved by SDS-PAGE. The 40-kDa GST-Jun band was excised and counted by liquid scintillation spectrometry. p54 kinase assays were performed in triplicate. Mean ± SD are shown. Another portion of extracts (1 ml) was subjected to Mono-Q chromatography; fractions were assayed for MAPK p42/44 activity as MBP kinase activity. A peak of stimulated MBP kinase activity was always detected eluting between 200 and 350 mM NaCl; total MAPK p42/44 activity was taken as MBP kinase activity in those fractions combined. 1 U p54 activity will transfer 1 pmol min^{-1} PO_4 from ATP to c-Jun. 1 U MAPK p42/44 will transfer 1 pmol min^{-1} PO_4 from ATP to MBP.

A central conundrum in the field of signal transduction is how agonists such as EGF and TNF-α can exert such different effects while apparently only activating one MAPK (the p42/p44 MAPK) pathway. The data in Figures 16 and 17, taken in combination with the known differences in SAPK and p42/p44 MAPK substrate specificity, suggest that signaling specificity may arise in part from the differential recruitment of signaling pathways such as the SAPK and p42/p44 MAPK pathways.

Whatever the identity of the elements that couple heat shock, protein synthesis inhibition, the cytokine receptor(s), and other cellular stresses to the SAPKs, the results presented here support the contention that the c-Jun polypeptide is a downstream target of the SAPKs. The SAPKs are a group of c-Jun N-terminal kinases. These kinases and members of the related superfamily focus a broad variety of regulatory signals, including phorbol esters, Ha-Ras, ultraviolet radiation

(Pulverer *et al.*; Smeal *et al.*; Adler *et al.*; Hibi *et al.*; Binétruy *et al.*; Devary *et al.*, (1992), *Cell*, 71:1081-1091), heat shock, protein synthesis inhibitors and cytokines into the phosphorylation of the c-Jun *trans* activation domain. The multiplicity of these c-Jun kinases and their activating inputs attest to the diversity of c-Jun function, and the complexity of its regulation. The role of c-Jun phosphorylation in the heat shock response and in the actions of TNF-α remain to be clarified. The consequences of c-Jun mediated transcriptional *trans* activation exhibit a great deal of cell specificity, and whether the outcome of MAPK- or SAPK-mediated c-Jun transactivation is mitogenesis, growth inhibition or a stable new phenotype resulting from altered gene expression is likely to depend on the array of signaling components acting in concert with AP-1.

Uses of the Invention

The extracellular signal regulated family of kinases are activated in response to different extracellular stimuli, and this specificity allows cells to diverge in their function in order to target a response to a given stimulus. The molecules of the invention can intervene in or stimulate a basic pathway modulating transcription of proteins that mediate an inflammatory or cell-stress response. Their use in the treatment and prevention of inflammation and the deleterious effects of hypoxia, heat stress, reperfusion injury, and other tissue insults may resolve many difficulties that arise with current therapies that rely on amelioration of the aftermath of damage instead of being able to redirect the course of the syndrome.

An additional use for these molecules may be in the upregulation of IL-2, which has proven useful in the

treatment of cancer. AP-1 activation is known to induce IL-2, and since c-Jun is a component of the AP-1 dimer, p54 phosphorylation of c-Jun would be expected to modulate AP-1 levels. p54 immunodepletion experiments have been shown to have a major effect on the phosphorylation of c-Jun.

A use for the p54 kinases is as a template for drug design, or a functional component for therapeutic drug assays. Many functional motifs of the molecules are already known (e.g., the ATP-binding site, sites of regulatory phosphorylation, c-Jun binding site, etc.) which, if an effective antagonist or agonist could be derived, could play an important role in therapies for conditions such as are listed above. Assays to screen for such drugs exist, and are described above. Monitoring the enzymatic activity following incubation with potentially therapeutic compounds such as was done with the drugs described herein would allow a simple, fast method to determine efficacy and dose-response for up or down regulation of the SAPKs. Large numbers of candidate compounds can be screened easily and evaluated for specificity, efficacy, and toxicity. Such information would allow rational evaluation of many drugs for their ability to up- or down-regulate cellular responses to physiological stresses, and be useful in clinical management of inflammation, ischemia, and many other stimuli that activate the SAPKs.

The molecules of the invention may be useful for reducing inflammation in such chronic disorders as autoimmune diseases or allergies, and in acute conditions such as anaphylactic shock, or soft tissue injury where swelling may aggravate the condition (e.g, around broken bones). Other uses include prophylactic treatment of patients about to undergo surgeries where there is a high likelihood of ischemia-reperfusion injury (e.g., vascular

surgery, organ transplants, etc.), or treatment of sepsis and fever.

For such conditions as mentioned above, a systemic application of the molecules of the invention, or antibodies derived from them, is generally desirable, although local application may be more appropriate in certain cases. Transport of the molecules to their site of action may be effected by, for example, liposome delivery systems, antisense technology, plasmid or retroviral vectors, or any of a number of other methods known in the art. The vehicle for application may be any excipient compatible with the molecules and the health of the patient.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: p54 STRESS-ACTIVATED PROTEIN KINASES

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US UNASSIGNED
(B) FILING DATE: 04-MAY-1994
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Phe Asp Thr Val Leu
        35                  40                  45

Gly Ile Asn Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
    50                  55                  60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Leu Lys Cys Val
65                  70                  75                  80

Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys
                85                  90                  95

Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp
            100                 105                 110

Ala Asn Leu Cys Gln Val Ile His Met Glu Leu Asp His Glu Arg Met
        115                 120                 125

Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly Ile Lys His Leu His Ser
    130                 135                 140

Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
145                 150                 155                 160

Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
                165                 170                 175

Cys Thr Asn Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr Arg
            180                 185                 190

Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp Ile
        195                 200                 205

Trp Ser Val Gly Cys Ile Met Ala Glu Met Val Leu His Lys Ser Cys
    210                 215                 220

Ser Pro Gly Arg Asp Tyr Ile Asp Gln Trp Asn Lys Val Ile Glu Gln
225                 230                 235                 240

Leu Gly Thr Pro Ser Ala Glu Phe Met Lys Lys Leu Gln Pro Thr Val
                245                 250                 255

Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr Pro Gly Ile Lys Phe Glu
            260                 265                 270

Glu Leu Phe Pro Asp Trp Ile Phe Pro Ser Glu Ser Glu Arg Asp Lys
        275                 280                 285

Ile Lys Thr Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
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Asp Pro Asp Lys Arg Ile Ser Val Asp Glu Ala Leu Arg His Pro Tyr
305                 310                 315                 320

Ile Thr Val Trp Tyr Asp Pro Ala Glu Ala Glu Ala Pro Pro Pro Gln
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Ile Tyr Asp Ala Gln Leu Glu Glu Arg Glu His Ala Ile Glu Glu Trp
            340                 345                 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Trp Glu Glu Arg Ser Lys
        355                 360                 365

Asn Gly Val Lys Asp Gln Pro Ser Asp Ala Ala Val Ser Ser Lys Ala
    370                 375                 380

Thr Pro Ser Gln Ser Ser Ser Ile Asn Asp Ile Ser Ser Met Ser Thr
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 1                5                  10                  15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Gln Leu Lys Pro Ile
            20                  25                  30

Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Phe Asp Thr Val Leu
        35                  40                  45

Gly Ile Asn Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
    50                  55                  60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Leu Lys Cys Val
65                  70                  75                  80

Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys
                85                  90                  95

Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp
            100                 105                 110

Ala Asn Leu Cys Gln Val Ile His Met Glu Leu Asp His Glu Arg Met
        115                 120                 125

Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly Ile Lys His Leu His Ser
    130                 135                 140

Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
145                 150                 155                 160
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Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
                165                 170                 175

Cys Thr Asn Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr Arg
            180                 185                 190

Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp Ile
        195                 200                 205

Trp Ser Val Gly Cys Ile Met Gly Glu Leu Val Lys Gly Cys Val Ile
    210                 215                 220

Phe Gln Gly Thr Asp His Ile Asp Gln Trp Asn Lys Val Ile Glu Gln
225                 230                 235                 240

Leu Gly Thr Pro Ser Ala Glu Phe Met Lys Lys Leu Gln Pro Thr Val
                245                 250                 255

Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr Pro Gly Ile Lys Phe Glu
            260                 265                 270

Glu Leu Phe Pro Asp Trp Ile Phe Pro Ser Glu Ser Glu Arg Asp Lys
        275                 280                 285

Ile Lys Thr Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
    290                 295                 300

Asp Pro Asp Lys Arg Ile Ser Val Asp Glu Ala Leu Arg His Pro Tyr
305                 310                 315                 320

Ile Thr Val Trp Tyr Asp Pro Ala Glu Ala Glu Ala Pro Pro Pro Gln
                325                 330                 335

Ile Tyr Asp Ala Gln Leu Glu Glu Arg Glu His Ala Ile Glu Glu Trp
            340                 345                 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Trp Glu Glu Arg Ser Lys
        355                 360                 365

Asn Gly Val Lys Asp Gln Pro Ser Asp Ala Ala Val Ser Ser Lys Ala
    370                 375                 380

Thr Pro Ser Gln Ser Ser Ser Ile Asn Asp Ile Ser Ser Met Ser Thr
385                 390                 395                 400

Glu His Thr Leu Ala Ser Asp Thr Asp Ser Ser Leu Asp Ala Ser Thr
                405                 410                 415

Gly Pro Leu Glu Gly Cys Arg
            420
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 426 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu Val Gly
1               5                   10                  15
```

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile
20 25 30

Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Val Leu
35 40 45

Asp Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
50 55 60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys Val
65 70 75 80

Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys
85 90 95

Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp
100 105 110

Ala Asn Leu Cys Gln Val Ile Gln Met Glu Leu Asp His Glu Arg Met
115 120 125

Ser Tyr Leu Leu Tyr Gln Met Leu Ser Ala Ile Lys His Leu His Ser
130 135 140

Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
145 150 155 160

Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
165 170 175

Gly Thr Ser Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr Arg
180 185 190

Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp Ile
195 200 205

Trp Ser Val Gly Cys Ile Met Gly Glu Met Val Arg His Lys Ile Leu
210 215 220

Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp Asn Lys Val Ile Glu Gln
225 230 235 240

Leu Gly Thr Pro Cys Pro Glu Phe Met Lys Lys Leu Gln Pro Thr Val
245 250 255

Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr Ala Gly Leu Thr Phe Pro
260 265 270

Lys Leu Phe Pro Asp Ser Leu Phe Pro Ala Asp Ser Glu His Asn Lys
275 280 285

Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
290 295 300

Asp Pro Ala Lys Arg Ile Ser Val Asp Asp Ala Leu Gln His Pro Tyr
305 310 315 320

Ile Asn Val Trp Tyr Asp Pro Ala Glu Val Glu Ala Pro Pro Pro Gln
325 330 335

Ile Tyr Asp Lys Gln Leu Asp Glu Arg Glu His Thr Ile Glu Glu Trp
340 345 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asn Ser Glu Glu Lys Thr Lys
355 360 365

```
Asn Gly Val Val Lys Gly Gln Pro Ser Pro Ser Gly Ala Ala Val Asn
    370                 375                 380

Ser Ser Glu Ser Leu Pro Pro Ser Ser Ser Val Asn Asp Ile Ser Ser
385                 390                 395                 400

Met Ser Thr Asp Gln Thr Leu Ala Ser Asp Thr Asp Ser Ser Leu Glu
                405                 410                 415

Ala Ser Ala Gly Pro Leu Gly Cys Cys Arg
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Lys Ser Lys Val Asp Asn Gln Phe Tyr Ser Val Glu Val Gly
1               5                   10                  15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile
            20                  25                  30

Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Val Leu
        35                  40                  45

Asp Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
    50                  55                  60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys Val
65                  70                  75                  80

Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys
                85                  90                  95

Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp
            100                 105                 110

Ala Asn Leu Cys Gln Val Ile Gln Met Glu Leu Asp His Glu Arg Met
        115                 120                 125

Ser Tyr Leu Leu Tyr Gln Met Leu Ser Ala Ile Lys His Leu His Ser
    130                 135                 140

Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
145                 150                 155                 160

Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
                165                 170                 175

Gly Thr Ser Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr Arg
            180                 185                 190

Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp Ile
        195                 200                 205

Trp Ser Val Gly Cys Ile Met Gly Glu Met Val Arg His Lys Ile Leu
    210                 215                 220
```

```
Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp Asn Lys Val Ile Glu Gln
225                 230                 235                 240

Leu Gly Thr Pro Cys Pro Glu Phe Met Lys Lys Leu Gln Pro Thr Val
                245                 250                 255

Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr Ala Gly Leu Thr Phe Pro
            260                 265                 270

Lys Leu Phe Pro Asp Ser Leu Phe Pro Ala Asp Ser Glu His Asn Lys
        275                 280                 285

Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
    290                 295                 300

Asp Pro Ala Lys Arg Ile Ser Val Asp Asp Ala Leu Gln His Pro Tyr
305                 310                 315                 320

Ile Asn Val Trp Tyr Asp Pro Ala Glu Val Glu Ala Pro Pro Pro Gln
                325                 330                 335

Ile Tyr Asp Lys Gln Leu Asp Glu Arg Glu His Thr Ile Glu Glu Trp
            340                 345                 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asn Ser Glu Glu Lys Thr Lys
        355                 360                 365

Asn Gly Val Val Lys Gly Gln Pro Ser Pro Ser Xaa Xaa Gly Ala Ala
    370                 375                 380

Val
385
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ser Arg Ser Lys Arg Asp Asn Asn Phe Tyr Ser Val Glu Ile Ala
1               5                   10                  15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile
            20                  25                  30

Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Ile Leu
        35                  40                  45

Glu Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
    50                  55                  60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys Val
65                  70                  75                  80

Asn His Lys Asn Ile Ile Gly Leu Leu Asn Val Phe Thr Pro Gln Lys
                85                  90                  95

Ser Leu Glu Glu Phe Gln Asp Val Tyr Ile Val Met Glu Leu Met Asp
            100                 105                 110
```

```
Ala Asn Leu Cys Gln Val Ile Gln Met Glu Leu Asp His Glu Arg Met
        115                 120                 125

Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly Ile Lys His Leu His Ser
    130                 135                 140

Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
145                 150                 155                 160

Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
                165                 170                 175

Gly Thr Ser Phe Met Met Thr Pro Tyr Val Val Thr Arg Tyr Tyr Arg
            180                 185                 190

Ala Pro Glu Val Ile Leu Gly Met Gly Tyr Lys Glu Asn Val Asp Leu
        195                 200                 205

Trp Ser Val Gly Cys Ile Met Gly Glu Met Val Cys Leu Lys Ile Leu
    210                 215                 220

Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp Asn Lys Val Ile Glu Gln
225                 230                 235                 240

Leu Gly Thr Pro Cys Pro Glu Phe Met Lys Lys Leu Gln Pro Thr Val
                245                 250                 255

Arg Thr Tyr Val Glu Asn Arg Pro Lys Tyr Ala Gly Tyr Ser Phe Glu
            260                 265                 270

Lys Leu Phe Pro Asp Val Leu Phe Pro Ala Asp Ser Glu His Asn Lys
        275                 280                 285

Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
    290                 295                 300

Asp Ala Ser Lys Arg Ile Ser Val Asp Glu Ala Leu Gln His Pro Tyr
305                 310                 315                 320

Ile Asn Val Trp Tyr Asp Pro Ser Glu Ala Glu Ala Pro Pro Pro Lys
                325                 330                 335

Ile Pro Asp Lys Gln Leu Asp Glu Arg Glu His Thr Ile Glu Glu Trp
            340                 345                 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Leu Glu Glu Arg Thr Lys
        355                 360                 365

Asn Gly Val Ile Arg Gly Gln Pro Ser Pro Leu Gly Ala Ala Val Ile
    370                 375                 380

Asn Gly Ser Gln His Pro Val Ser Ser Pro Ser Val Asn Asp Met Ser
385                 390                 395                 400

Ser Met Ser Thr Asp Pro Thr Leu Ala Ser Asp
                405                 410
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Asp Ala Ala Val Ser Ser Lys Ala Thr Pro Ser Gln Ser Ser Ser
1 5 10 15

Ile

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Ala Ala Val Asn Ser Ser Glu Ser Leu Pro Pro Ser Ser Ser Val
1 5 10 15

Gln Pro Ser Pro Ser
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser Pro Leu Gly Ala Ala Val Ile Asn Gln Ser Gln His Pro Val Ser
1 5 10 15

Ser Pro Ser Val
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGATTCCTGT ATGACACTAC ATCATGAGTG ACAGTAAAAG CGATGGCCAG TTTTACAGTG 60

TGCAAGTGGC AGACTCAACT TTCACTGTTC TAAAACGTTA CCAGCAGTTG AAACCAATTG 120

GCTCTGGAGC CCAAGGAATT GTTTGTGCTG CTTTTGATAC AGTTCTTGGA ATAAATGTTG 180
CTGTCAAGAA GTTAAGTCGT CCTTTTCAGA ACCAAACGCA TGCAAAGAGA GCCTACCGTG 240
AACTTGTCCT CCTAAAGTGT GTCAATCATA AAAATATAAT TAGCTTGTTA AATGTGTTCA 300
CACCACAAAA AACGCTAGAA GAATTCCAAG ATGTGTACTT GGTTATGGAG TTAATGGACG 360
CTAACTTATG TCAGGTTATT CATATGGAGC TGGACCATGA AAGAATGTCA TACCTCCTCT 420
ACCAGATGCT TTGTGGCATT AAGCACCTGC ATTCAGCTGG CATAATTCAT AGGGATTTGA 480
AGCCTAGCAA CATTGTAGTA AAATCAGACT GTACTCTCAA GATCCTTGAC TTTGGCCTGG 540
CACGGACAGC CTGTACCAAC TTTATGATGA CTCCCTATGT GGTAACTCGC TACTATCGGG 600
CTCCAGAAGT CATCCTGGGC ATGGGCTACA AGGAGAATGT GGACATCTGG TCTGTCGGGT 660
GCATCATGGC AGAAATGGTC CTCCATAAAT CCTGTTCCCC AGGAAGAGAC TATATTGATC 720
AATGGAATAA AGTTATTGAA CAGCTAGGAA CACCATCCGC AGAGTTCATG AAGAAACTTC 780
AGCCAACTGT AAGGAATTAT GTGGAAAACA GACCAAAGTA CCCTGGAATC AAATTTGAAG 840
AGCTCTTTCC AGATTGGATA TTTCCGTCAG AATCCGAACG AGACAAAATA AAAACAAGTC 900
AAGCCAGAGA TCTGTTATCG AAAATGTTAG TGATTGATCC GGACAAGCGG ATCTCTGTGG 960
ACGAAGCCTT GCGCCACCCG TATATTACTG TTTGGTATGA CCCCGCTGAA GCAGAAGCGC 1020
CACCACCTCA AATTTATGAT GCCCAGTTGG AAGAAAGAGA GCATGCGATT GAAGAGTGGA 1080
AAGAACTAAT TTACAAAGAA GTGATGGACT GGGAAGAAAG AAGCAAGAAT GGGGTGAAAG 1140
ACCAGCCTTC AGATGCAGCA GTAAGCAGCA AGGCTACTCC TTCTCAGTCG TCATCCATCA 1200
ATGACATCTC ATCCATGTCC ACTGAGCACA CCCTGGCCTC AGACACAGAC AGCAGTCTCG 1260
ATGCCTCAAC CGGACCCCTG GAAGGCTGCC GATGAAACCT CGCAGATGGC GCACTTGTCT 1320
GTGAAGGACT CTGGCTTCCA TGGCCCTGAG CACATGGGAG CTGGTGGAAC AAATCAAGAA 1380
GCTCCATGTT CTGCATGTAA GAAACACGAC GCCTTGCCCC CACTCAGTTC CAGTAGGATT 1440
GCCTGCGTAG ACTGTAACAT GAGGCAGACG ATGTCTGGAG AAAAAGTACA AACCACACTG 1500
TTAGAAATTT TGTTCAAGAT CATTCAGGTG AGCAATTAGA ATAGCCGAGT TCTTTTCAAG 1560
TCGTGTGGTG TCCTTGGTGA CAGATCATGT GTAACTGTGG GGACTCGTAT GCATGTGACC 1620
ACAAATGCTT GCTTGAACTT GCCCATGTAG CACTTTGGGA ATCAGTATTT AAATGCCAAA 1680
TAATCTTCCA GGTAGTTCTG CTTCTAGAAT AATCTCTTAA TCCTCTTTAG TAATTTGGTG 1740
TCTGTCCACA AAAAAATAGA TTATGTGTGT ATGAATTGGC CACTATCATA TTATCATATT 1800
TTACCCACTT TTATGGTATG ATTTATTCTG TCTTTTGTAT TTCAGAAGGA ATATAATTAA 1860
ATTTATTTAA TAAATAAAAC TACAGCTTTT CTTAAATTTG TGATGTTTTA GGCTGAGAAT 1920
TACCACTGCT TTATATCGAC ACTCTGTGTC CTTTAAACTG CCCACTATGG GAAACTTTAC 1980
GTACAGCTTT CTGCATGACA AAGTTCCAAG TTGTATTTCA CTCTGCTTAA CGACTTATGT 2040
CACCTTGAAT CCTGACCACA CATTTCCTTT TTCTTGGTCC TCTGAACTTG GATCTAGAAT 2100
CCCTCAGAGA ACTTCACCTT CTTTATCACA AAGCACCCCA TCTCAGTAGA ATGAATCGGC 2160

AGATTCCTGA GCCCCGCTGC CTAATGTAGA GCTGACAGGG TGGCTTCCCC AGAACGGTGG 2220

GTGGGTGCAT CCTTCCCTGA GCCCACCCAT CCTTTGCTCC CCTCTCTTTA TTTAAGGTGA 2280

AAGGTGATTG GGTCTCATAG CCTTTCCTTT TGTAGCATTG CCTAACTTGT CTTTCTCACT 2340

GACAGAAGCC ACCACGTCCA GCCAGAGCAC ATGGTCTCTT AGGAGACCGG GCTTACTTAC 2400

CATGCATGTT TGCTGCTGTC CTTTTCCATT TTGTGGAGGC ATTTCCTTTT TCTAAGGGAA 2460

TTCCTCAGAT GTTCTAGAAA CATTCAGAAG AACGCAGAAG AAATATTCTA GAGAATTGGG 2520

GGTTCATTCT TGAATATTTT CTGATTTAAA ACTGCTCACC TGAAATTGAT ACTTTCAGAT 2580

CCTGATCTTG TAAATTACTC GAGATTTGGT AAGATGCTGA GTTCTCTGT 2629

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGATTCCTGT ATGACACTAC ATCATGAGTG ACAGTAAAAG CGATGGCCAG TTTTACAGTG 60

TGCAAGTGGC AGACTCAACT TTCACTGTTC TAAAACGTTA CCAGCAGTTG AAACCAATTG 120

GCTCTGGAGC CCAAGGAATT GTTTGTGCTG CTTTTGATAC AGTTCTTGGA ATAAATGTTG 180

CTGTCAAGAA GTTAAGTCGT CCTTTTCAGA ACCAAACGCA TGCAAAGAGA GCCTACCGTG 240

AACTTGTCCT CCTAAAGTGT GTCAATCATA AAAATATAAT TAGCTTGTTA AATGTGTTCA 300

CACCACAAAA AACGCTAGAA GAATTCCAAG ATGTGTACTT GGTTATGGAG TTAATGGACG 360

CTAACTTATG TCAGGTTATT CATATGGAGC TGGACCATGA AAGAATGTCA TACCTCCTCT 420

ACCAGATGCT TTGTGGCATT AAGCACCTGC ATTCAGCTGG CATAATTCAT AGGGATTTGA 480

AGCCTAGCAA CATTGTAGTA AAATCAGACT GTACTCTCAA GATCCTTGAC TTTGGCCTGG 540

CACGGACAGC CTGTACCAAC TTTATGATGA CTCCCTATGT GGTAACTCGC TACTATCGGG 600

CTCCAGAAGT CATCCTGGGC ATGGGCTACA AGGAGAATGT TGATATCTGG TCAGTGGGTT 660

GCATCATGGG AGAGCTGGTG AAAGGTTGTG TGATATTCCA AGGTACTGAC CATATTGATC 720

AATGGAATAA AGTTATTGAA CAGCTAGGAA CACCATCCGC AGAGTTCATG AAGAAACTTC 780

AGCCAACTGT AAGGAATTAT GTGGAAAACA GACCAAAGTA CCCTGGAATC AAATTTGAAG 840

AGCTCTTTCC AGATTGGATA TTTCCGTCAG AATCCGAACG AGACAAAATA AAAACAAGTC 900

AAGCCAGAGA TCTGTTATCG AAAATGTTAG TGATTGATCC GGACAAGCGG ATCTCTGTGG 960

ACGAAGCCTT GCGCCACCCG TATATTACTG TTTGGTATGA CCCCGCTGAA GCAGAAGCGC 1020

CACCACCTCA AATTTATGAT GCCCAGTTGG AAGAAAGAGA GCATGCGATT GAAGAGTGGA 1080

AAGAACTAAT TTACAAAGAA GTGATGGACT GGGAAGAAAG AAGCAAGAAT GGGGTGAAAG 1140

ACCAGCCTTC AGATGCAGCA GTAAGCAGCA AGGCTACTCC TTCTCAGTCG TCATCCATCA 1200

ATGACATCTC ATCCATGTCC ACTGAGCACA CCCTGGCCTC AGACACAGAC AGCAGTCTCG 1260

ATGCCTCAAC CGGACCCCTG GAAGGCTGCC GATGAAACCT CGCAGATGGC GCACTTGTCT 1320

GTGAAGGACT CTGGCTTCCA TGGCCCTGAG CACATGGGAG CTGGTGGAAC AAATCAAGAA 1380

GCTCCATGTT CTGCATGTAA GAAACACGAC GCCTTGCCCC CACTCAGTTC CAGTAGGATT 1440

GCCTGCGTAG ACTGTAACAT GAGGCAGACG ATGTCTGGAG AAAAAGTACA AACCACACTG 1500

TTAGAAATTT TGTTCAAGAT CATTCAGGTG AGCAATTAGA ATAGCCGAGT TCTTTTCAAG 1560

TCGTGTGGTG TCCTTGGTGA CAGATCATGT GTAACTGTGG GGACTCGTAT GCATGTGACC 1620

ACAAATGCTT GCTTGAACTT GCCCATGTAG CACTTTGGGA ATCAGTATTT AAATGCCAAA 1680

TAATCTTCCA GGTAGTTCTG CTTCTAGAAT AATCTCTTAA TCCTCTTTAG TAATTTGGTG 1740

TCTGTCCACA AAAAAATAGA TTATGTGTGT ATGAATTGGC CACTATCATA TTATCATATT 1800

TTACCCACTT TTATGGTATG ATTTATTCTG TCTTTTGTAT TTCAGAAGGA ATATAATTAA 1860

ATTTATTTAA TAAATAAAAC TACAGCTTTT CTTAAATTTG TGATGTTTTA GGCTGAGAAT 1920

TACCACTGCT TTATATCGAC ACTCTGTGTC CTTTAAACTG CCCACTATGG GAAACTTTAC 1980

GTACAGCTTT CTGCATGACA AAGTTCCAAG TTGTATTTCA CTCTGCTTAA CGACTTATGT 2040

CACCTTGAAT CCTGACCACA CATTTCCTTT TTCTTGGTCC TCTGAACTTG GATCTAGAAT 2100

CCCTCACAGA ACTTCACCTT CTTTATCACA AAGCACCCCA TCTCAGTAGA ATGAATCGGC 2160

AGATTCCTGA GCCCCGCTGC CTAATGTAGA GCTGACAGGG TGGCTTCCCC AGAACGGTGG 2220

GTGGGTGCAT CCTTCCCTGA GCCCACCCAT CCTTTGCTCC CCTCTCTTTA TTTAAGGTGA 2280

AAGGTGATTG GGTCTCATAG CCTTTCCTTT TGTAGCATTG CCTAACTTGT CTTTCTCACT 2340

GACAGAAGCC ACCACGTCCA GCCAGAGCAC ATGGTCTCTT AGGAGACCGG GCTTACTTAC 2400

CATGCATGTT TGCTGCTGTC CTTTTCCATT TTGTGGAGGC ATTTCCTTTT TCTAAGGGAA 2460

TTCCTCAGAT GTTCTAGAAA CATTCAGAAG AACGCAGAAG AAATATTCTA GAGAATTGGG 2520

GGTTCATTCT TGAATATTTT CTGATTTAAA ACTGCTCACC TGAAATTGAT ACTTTCAGAT 2580

CCTGATCTTG TAAATTACTC GAGATTTGGT AAGATGCTGA GTTCTCTGT 2629

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1975 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCTCCTTAT TCCGGTTTGG AATGTGGCTA ATGAAAGCCC AGTAGGAGGA TTTCTGGGGC 60

AAACAGGTGG ACCAGGATCC TGGTTCTCAG GCACGGAATG GCTATTGTGA GAGCGCCACC 120

AGCAGGACCA TCGCAGATCT TGGTTATGGC TGCTCACGCA AGAGGCTGTT GATGTAGACC 180

CCCTTTCCCG TAGATGAGAA ATCACACGAG CAGTGGTATT TATGAGCCTC CATTTCTTAT 240

ACTACTGCAG TGAACCAACC TTGGATGTGA AAATTGCCTT TTGTCAGGTG TGTGTTCCTT 300

ACAGGTAAAA CAAAGGGATT CGACAAACAC GTGGATGTGT CTTCTGTTGT CAAACATTAC 360

AACATGAGCA AAAGCAAGGT AGATAACCAG TTCTACAGTG TGGAAGTGGG AGACTCAACC 420

TTCACAGTTC TAAAGCGCTA CCAGAACCTG AAGCCGATCG GCTCTGGGGC TCAGGGAATA 480

GTTTGTGCTG CGTATGACGC TGTCCTCGAC AGAAATGTGG CCATTAAGAA GCTCAGCAGA 540

CCCTTCCAGA ACCAAACTCA TGCCAAGAGG GCTTACCGGG AGCTGGTCCT CATGAAGTGT 600

GTGAACCATA AAAACATTAT TAGCTTATTA AATGTCTTTA CACCCCAGAA AACACTGGAG 660

GAGTTCCAAG ATGTTTACTT AGTGATGGAA CTGATGGACG CCAACTTGTG TCAGGTGATT 720

CAGATGGAGC TGGACCACGA GCGGATGTCG TACTTGCTGT ACCAGATGCT GTCGGCGATC 780

AAACACCTCC ACTCCGCTGG GATCATCCAC AGGGACTTAA AACCCAGTAA CATCGTAGTC 840

AAGTCTGATT GCACACTGAA AATCCTGGAC TTTGGACTGG CCAGGACAGC GGGCACAAGC 900

TTCATGATGA CTCCGTATGT GGTGACGAGA TATTACAGAG CCCCCGAGGT CATCCTGGGC 960

ATGGGCTACA AGGAGAACGT GGACATATGG TCTGTGGGCT GCATCATGGG AGAAATGGTT 1020

CGTCACAAAA TCCTCTTTCC CGGAAGGGAC TATATTGACC AGTGGAACAA AGTCATAGAG 1080

CAGCTAGGAA CTCCGTGTCC AGAATTCATG AAGAAATTGC AGCCCACCGT CAGAAACTAC 1140

GTGGAGAACC GGCCCAAGTA TGCAGGCCTC ACCTTCCCCA AGCTCTTTCC AGATTCCCTC 1200

TTCCCAGCGG ATTCCGAGCA CAATAAACTT AAAGCCAGCC AAGCCAGGGA CTTGTTGTCA 1260

AAGATGTTAG TGATTGACCC AGCGAAGAGG ATATCGGTGG ATGACGCATT GCAGCATCCG 1320

TACATCAACG TTTGGTACGA CCCTGCTGAA GTGGAGGCGC CTCCGCCTCA GATATATGAC 1380

AAGCAATTGG ATGAAAGGGA GCACACCATC GAAGAATGGA AAGAACTCAT CTACAAGGAA 1440

GTAATGAACT CAGAAGAGAA GACTAAGAAC GGCGTAGTCA AAGGCCAGCC CTCACCTTCA 1500

GGTGCAGCAG TGAACAGCAG TGAGAGTCTC CCTCCATCCT CATCTGTCAA CGACATCTCC 1560

TCCATGTCCA CCGACCAGAC CCTCGCATCC GACACTGACA GCAGCCTGGA AGCCTCGGCG 1620

GGACCGCTGG GTTGTTGCAG GTGACTAGCC GCCTGCCTGC GAAACCCAGC GTTCTTCAGG 1680

AGATGACGCC ATGATAGAAC ACAGCGCACA TGCACACACA CAGAGCTTGT ACACACACAC 1740

ACACACACAC ACACACGCAC GCACGCACGC ACGCAAGCAC GCACGCACGC ACAAATGCAC 1800

TCACGCAATG TCAAGAAAAA AAAAAGTAGC GAGAGAGAGC GAGAGAGCCA ACGTAAAACT 1860

AAGTTAAATC TTTCTGCGTG CTTCTCCAGA GTTCTGTATC GCAGCTGAGC TGAAATGTAT 1920

ACTTAACTTC TAGTCGCGCT CGCTCGACTT TGGTCTCCCT CCGGCAGTGC TTACT 1975

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1986 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCTATCCCT CCTTATTCCG GTTTGGAATG TGGCTAATGA AAGCCCAGTA GGAGGATTTC 60

TGGGGCAAAC AGGTGGACCA GGATCCTGGT TCTCAGGCAC GGAATGGCTA TTGTGAGAGC 120

GCCACCAGCA GGACCATCGC AGATCTTGGT TATGGCTGCT CACGCAAGAG GCTGTTGATG 180

TAGACCCCCT TTCCCGTAGA TGAGAAATCA CACGAGCAGT GGTATTTATG AGCCTCCATT 240

TCTTATACTA CTGCAGTGAA CCAACCTTGG ATGTGAAAAT TGCCTTTTGT CAGGTGTGTG 300

TTCCTTACAG GTAAAACAAA GGGATTCGAC AAACACGTGG ATGTGTCTTC TGTTGTCAAA 360

CATTACAACA TGAGCAAAAG CAAGGTAGAT AACCAGTTCT ACAGTGTGGA AGTGGGAGAC 420

TCAACCTTCA CAGTTCTAAA GCGCTACCAG AACCTGAAGC CGATCGGCTC TGGGGCTCAG 480

GGAATAGTTT GTGCTGCGTA TGACGCTGTC CTCGACAGAA ATGTGGCCAT TAAGAAGCTC 540

AGCAGACCCT TCCAGAACCA AACTCATGCC AAGAGGGCTT ACCGGGAGCT GGTCCTCATG 600

AAGTGTGTGA ACCATAAAAA CATTATTAGC TTATTAAATG TCTTTACACC CCAGAAAACA 660

CTGGAGGAGT TCCAAGATGT TTACTTAGTG ATGGAACTGA TGGACGCCAA CTTGTGTCAG 720

GTGATTCAGA TGGAGCTGGA CCACGAGCGG ATGTCGTACT TGCTGTACCA GATGCTGTCG 780

GCGATCAAAC ACCTCCACTC CGCTGGGATC ATCCACAGGG ACTTAAAACC CAGTAACATC 840

GTAGTCAAGT CTGATTGCAC ACTGAAAATC CTGGACTTTG GACTGGCCAG GACAGCGGGC 900

ACAAGCTTCA TGATGACTCC GTATGTGGTG ACGAGATATT ACAGAGCCCC CGAGGTCATC 960

CTGGGCATGG GCTACAAGGA GAACGTGGAC ATATGGTCTG TGGGCTGCAT CATGGGAGAA 1020

ATGGTTCGTC ACAAAATCCT CTTTCCCGGA AGGGACTATA TTGACCAGTG GAACAAAGTC 1080

ATAGAGCAGC TAGGAACTCC GTGTCCAGAA TTCATGAAGA AATTGCAGCC CACCGTCAGA 1140

AACTACGTGG AGAACCGGCC CAAGTATGCA GGCCTCACCT TCCCCAAGCT CTTTCCAGAT 1200

TCCCTCTTCC CAGCGGATTC CGAGCACAAT AAACTTAAAG CCAGCCAAGC CAGGGACTTG 1260

TTGTCAAAGA TGTTAGTGAT TGACCCAGCG AAGAGGATAT CGGTGGATGA CGCATTGCAG 1320

CATCCGTACA TCAACGTTTG GTACGACCCT GCTGAAGTGG AGGCGCCTCC GCCTCAGATA 1380

TATGACAAGC AATTGGATGA AAGGGAGCAC ACCATCGAAG AATGGAAAGA ACTCATCTAC 1440

AAGGAAGTAA TGAACTCAGA AGAGAAGACT AAGAACGGCG TAGTCAAAGG CCAGCCCTCA 1500

CCTTCAGCAC AGGTGCAGCA GTGAACAGCA GTGAGAGTCT CCCTCCATCC TCATCTGTCA 1560

ACGACATCTC CTCCATGTCC ACCGACCAGA CCCTCGCATC CGACACTGAC AGCAGCCTGG 1620

AAGCCTCGGC GGGACCGCTG GGTTGTTGCA GGTGACTAGC CGCCTGCCTG CGAAACCCAG 1680

CGTTCTTCAG GAGATGACGC CATGATAGAA CACAGCGCAC ATGCACACAC ACAGAGCTTG 1740

TACACACAC. CACACACACA CACACACGCA CGCACGCACG CACGCAAGCA CGCACGCACG 1800

CACAAATGCA CTCACGCAAT GTCAAGAAAA AAAAAAGTAG CGAGAGAGAG CGAGAGAGCC 1860

AACGTAAAAC TAAGTTAAAT CTTTCTGCGT GCTTCTCCAG AGTTCTGTAT CGCAGCTGAG 1920

CTGAAATGTA TACTTAACTT CTAGTCGCGC TCGCTCGACT TTGGTCTCCC TCCGGCAGTG 1980

CTTACT 1986

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCGGCCGAGC GCGGGACGTT GCGGCCGAAA CGCGGAGCCG CGAGCAGGAT TAAGTAGCGG 60

CCCGGCCACC GGCACGGCGC CGCTCTCCGC TACTGGCTTC CAGGTCTCCG TTGGCTGCAC 120

TGCCGGCCGG TTGTTGAATA TTTGGATGAA GCCATTAGAC TAATTGCTTG CCATCATGAG 180

CAGAAGTAAA CGTGACAACA ATTTTTATAG TGTAGAGATC GCAGATTCTA CATTCACAGT 240

CCTAAAACGA TACCAGAACT TAAAGCCTAT AGGCTCAGGA GCTCAAGGAA TAGTGTGTGC 300

AGCTTATGAT GCTATTCTTG AAAGAAATGT TGCAATCAAG AAGCTCAGCC GGCCATTTCA 360

GAATCAGACC CATGCTAAGC GAGCCTACCG AGAACTAGTT CTTATGAAGT GTGTTAATCA 420

CAAAAATATA ATTGGCCTTT TGAATGTTTT CACACCACAG AAATCCCTAG AAGAATTTCA 480

AGATGTTTAC ATAGTCATGG AGCTCATGGA TGCAAATCTT TGCCAAGTGA TTCAGATGGA 540

GTTAGATCAT GAAAGAATGT CCTACCTTCT CTATCAAATG CTGTGTGGAA TCAAGCACCT 600

TCACTCTGCT GGAATTATTC ATCGGGACTT AAAGCCTAGT AATATAGTAG TCAAATCAGA 660

CTGCACTTTG AAGATTCTTG ATTTTGGACT GGCAAGGACT GCAGGAACGA G[illegible]GAT 720

GACGCCTTAC GTGGTAACTC GTTACTACAG AGCACCAGAG GTCATTCTCC GCATGGGCTA 780

CAAGGAGAAC GTGGATTTAT GGTCTGTGGG GTGCATTATG GGAGAAATGG TTTGCCTCAA 840

AATCCTCTTT CCAGGAAGGG ACTATATTGA TCAGTGGAAT AAAGTTATTG AACAGCTCGG 900

AACACCTTGT CCTGAATTCA TGAAGAAACT ACAACCAACA GTAAGGACTT ACGTTGAAAA 960

CAGACCTAAG TACGCTGGCT ATAGCTTTGA GAAACTGTTT CCTGATGTGC TTTTCCCAGC 1020

TGACTCAGAA CATAACAAAC TTAAAGCCAG TCAGGCGAGA GATTTGTTAT CTAAAATGCT 1080

GGTGATAGAT GCGTCCAAAA GGATCTCCGT AGACGAAGCT CTCCAGCACC CGTACATCAA 1140

CGTCTGGTAT GATCCTTCAG AAGCAGAGGC CCCACCACCA AAGATCCCTG ACAAGCAGTT 1200

AGATGAAAGG GAGCACACAA TAGAGGAGTG GAAAGAACTG ATATACAAGG AGGTCATGGA 1260

TTTGGAGGAG CGAACTAAGA ATGGCGTCAT AAGAGGGCAG CCGTCTCCTT TAGGTGCAGC 1320

AGTGATCAAT GGCTCTCAGC ATCCGGTCTC TTCGCCGTCT GTCAATGACA TGTCTTCAAT 1380

GTCCACAGAT CCGACTCTGG CCTCGGAT 1408

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAGATGC CAGTAAGCAG CAAGGCTACT CCTTCTCAGT CGTCATCCAT C 51

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGCCCTCAC CTTCAGGTGC AGCAGTGAAC AGCAGTGAGA GTCTCCCTCC ATCCTCATCT 60

GTC 63

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTCCTTTAG GTGCAGCAGT GATCAATGGC TCTCAGCATC CGGTCTCTTC GCCGTCTGTC 60

What is claimed is:

1. A recombinant p54 stress-activated protein kinase polypeptide or biologically active fragment thereof at least 10 amino acids in length.

2. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence substantially identical to SEQ ID NO: 1.

3. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence at least 95% identical to SEQ ID NO: 1.

4. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence substantially identical to SEQ ID NO: 2.

5. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence at least 95% identical to SEQ ID NO: 2.

6. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence substantially identical to SEQ ID NO: 3.

7. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence at least 95% identical to SEQ ID NO: 3.

8. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence substantially identical to SEQ ID NO: 4.

9. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence at least 95% identical to SEQ ID NO: 4.

10. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence substantially identical to SEQ ID NO: 5.

11. The polypeptide of claim 1, wherein said polypeptide or fragment thereof is derived from an amino acid sequence at least 95% identical to SEQ ID NO: 5.

12. The polypeptide of claim 1 wherein said polypeptide is derived from a mammal.

13. The polypeptide of claim 12 wherein said mammal is a rat.

14. The polypeptide of claim 12 wherein said mammal is a human.

15. The polypeptide of claim 1 wherein said polypeptide or fragment thereof is useful for producing antibodies which specifically bind to a p54 stress-activated protein kinase.

16. The antibody of claim 15 wherein said polypeptide fragment is chosen from the group consisting of SEQ ID NOs: 6, 7, and 8.

17. A DNA and its degenerate variants which encode a p54 stress-activated protein kinase polypeptide, or a biologically active fragment thereof at least 30 nucleotides in length.

18. The DNA of claim 17 comprising a nucleotide sequence encoding a p54αI polypeptide at least 90% identical to SEQ ID NO 9.

19. The DNA of claim 17 comprising a nucleotide sequence encoding a p54αII polypeptide at least 90% identical to SEQ. ID NO.: 10.

20. The DNA of claim 17 comprising a nucleotide sequence encoding a p54βI polypeptide at least 90% identical to SEQ. ID NO.: 11.

21. The DNA of claim 17 comprising a nucleotide sequence encoding a p54βII polypeptide at least 90% identical to SEQ. ID NO.: 12.

22. The DNA of claim 17 comprising a nucleotide sequence encoding a p54γ polypeptide at least 90% identical to SEQ. ID NO.: 13.

23. The DNA of claim 17 wherein said fragment is chosen from the group consisting of SEQ ID NOs: 14, 15, and 16.

24. The DNA of claim 17 wherein said DNA is derived from a mammal.

25. The DNA of claim 24 wherein said mammal is a rat.

26. The DNA of claim 24 wherein said mammal is a human.

27. A DNA which hybridizes under stringent conditions to one or more of the DNAs chosen from the group SEQ ID NO. 9, 10, 11, 12, and 13.

28. A method of screening potentially therapeutic compounds comprising the steps of

a) treating cultured cells by applying said compounds and stress-activated protein kinase-activating stimuli,

b) preparing cytoplasmic extracts of said treated cells, then

c) assaying the isolated recombinant stress-activated protein kinases for c-Jun kinase activity.

29. The method of claim 28 wherein said cultured cells are selected from the group consisting of human cell lines HepG2, CCD-18Co, U937, and HT-29.

30. A method of screening potentially therapeutic compounds comprising the steps of

a) treating cultured cells by applying said compounds,

b) preparing cytoplasmic extracts of said treated cells,

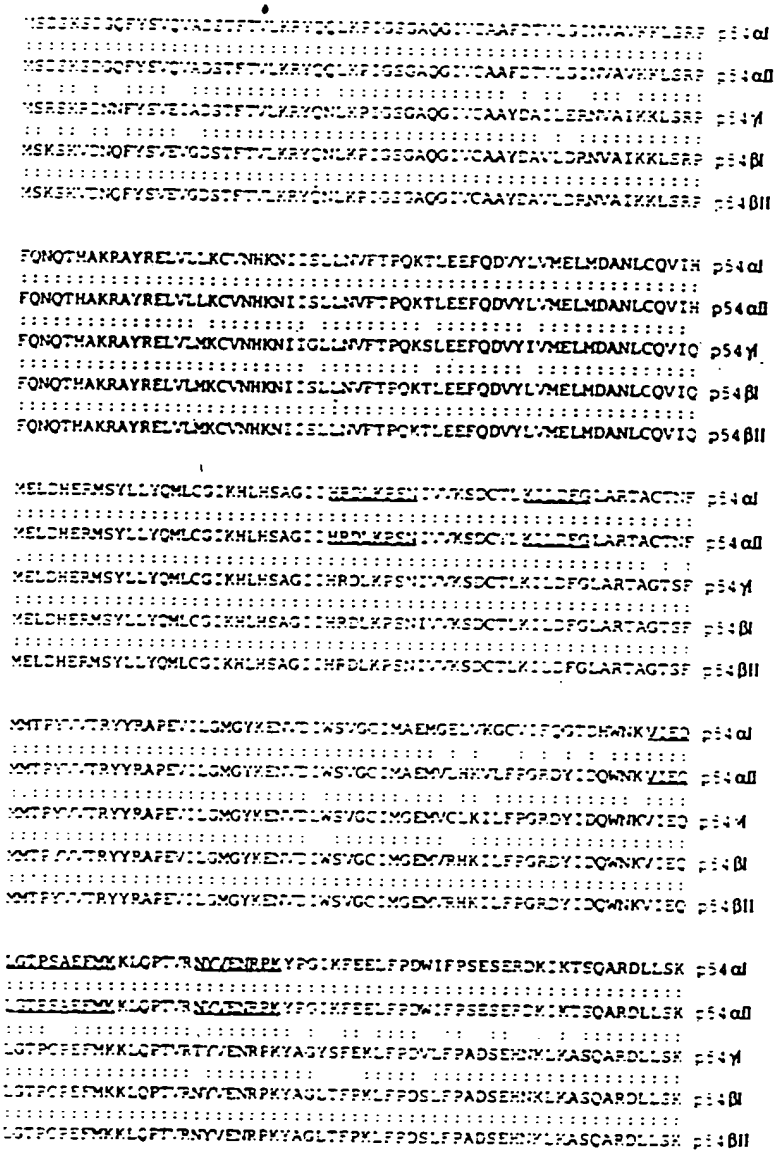
c) combining said extracts with an inactive recombinant stress-activated protein kinase, and then

d) assaying the isolated recombinant stress-activated protein kinases for c-Jun kinase activity.

31. The method of claim 30 wherein said cultured cells are selected from the group consisting of human cell lines HepG2, CCD-18Co, U937, and HT-29.

32. The method of claim 30 wherein said cultured cells have been treated previously or concurrently with stress-activated protein kinase-activating stimuli.

FIG. 1



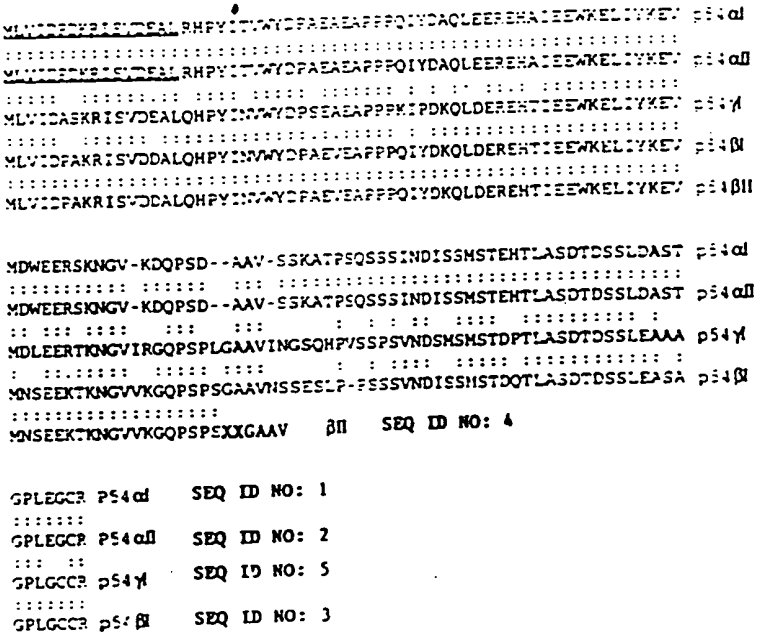

p54αI
p54αII
p54γI
p54βI
p54βII
p54αI
p54αII
p54γI
p54βI
βII SEQ ID NO: 4
GPLEGCR p54αI SEQ ID NO: 1
GPLEGCR p54αII SEQ ID NO: 2
GPLGCCR p54γI SEQ ID NO: 5
GPLGCCR p54βI SEQ ID NO: 3

FIG. 2A

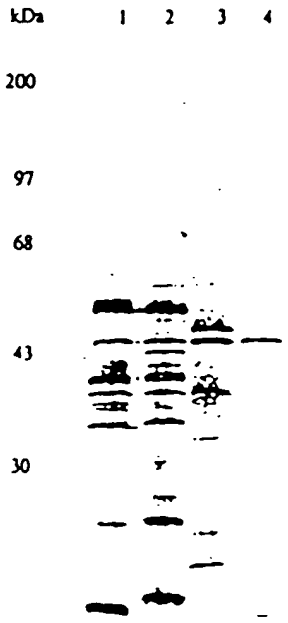
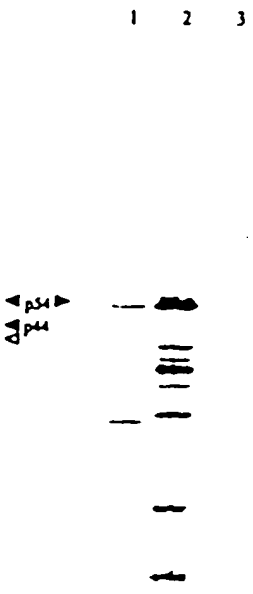


FIG. 2B



Immunodepletion of p54s (SAPKs) removes >60% of GST-c-Jun kinase from extracts of TNF-α-treated Hep G2 cells

FIG. 3A

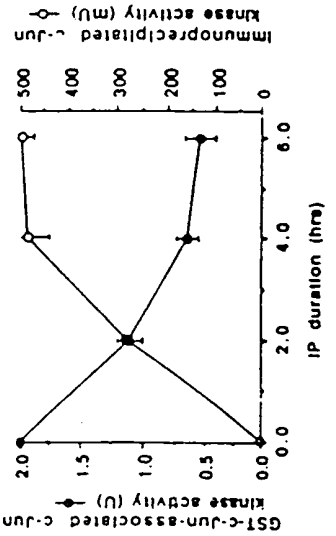
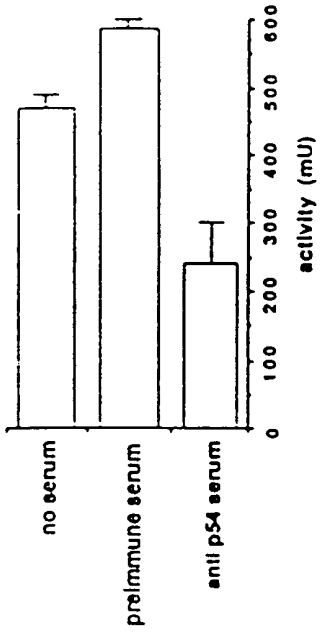


FIG. 3B



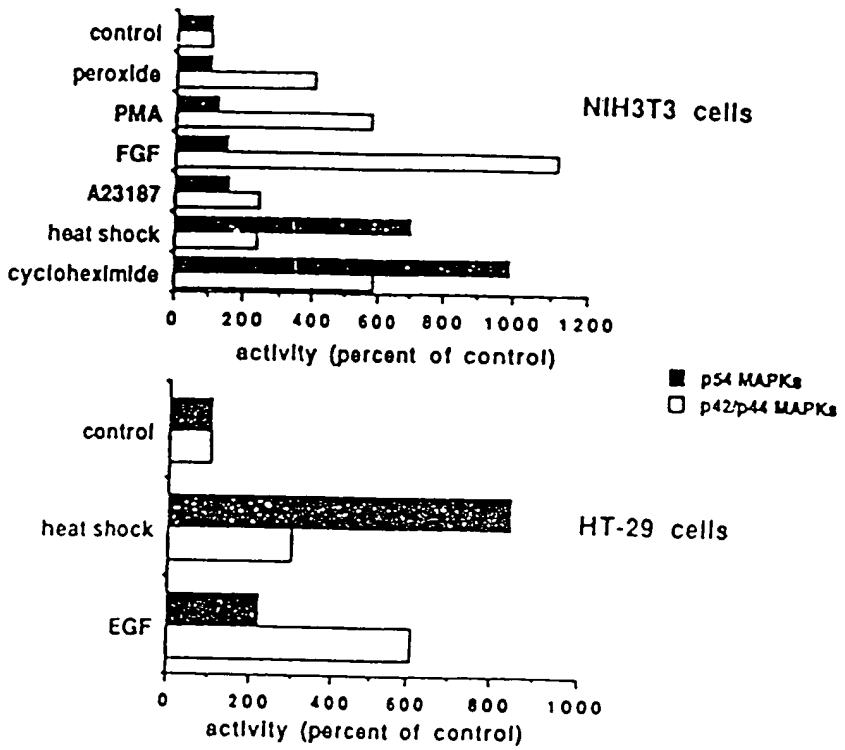

control
peroxide
PMA
FGF
A23187
heat shock
cycloheximide
0
200
400
600
800
1000
1200
activity (percent of control)
NIH3T3 cells
p54 MAPKs
p42/p44 MAPKs
control
heat shock
EGF
0
200
400
600
800
1000
activity (percent of control)
HT-29 cells

FIG. 4A

FIG. 4B

FIG. 5A

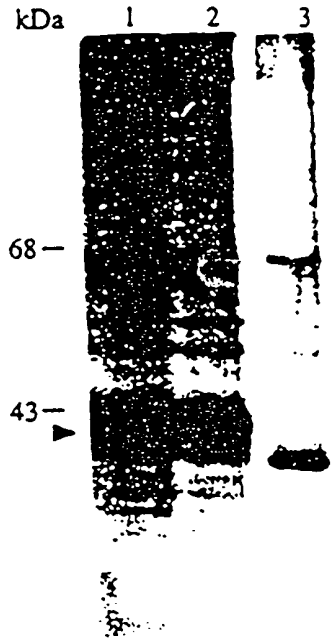
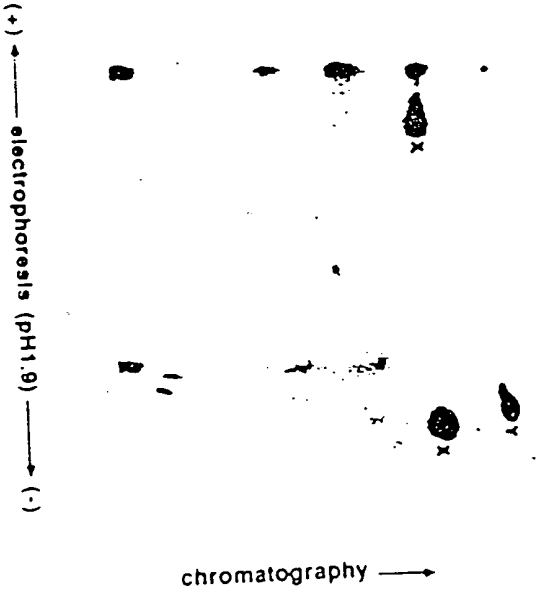


FIG. 5B



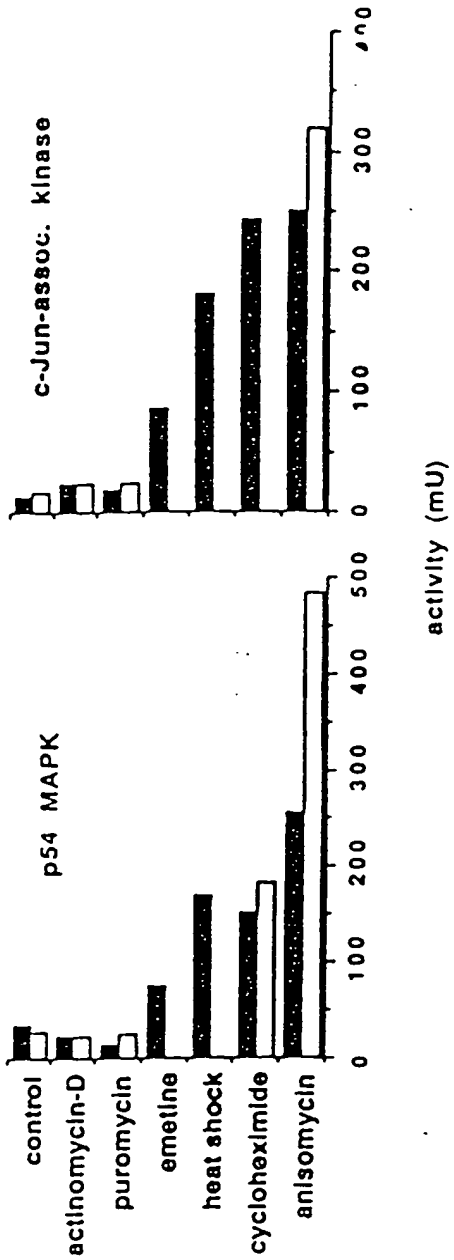

c-Jun-assoc. kinase
p54 MAPK
0
100
200
300
400
500
activity (mU)
control
actinomycin-D
puromycin
emetine
heat shock
cycloheximide
anisomycin

FIG. 6A

FIG. 6B

FIG. 7A

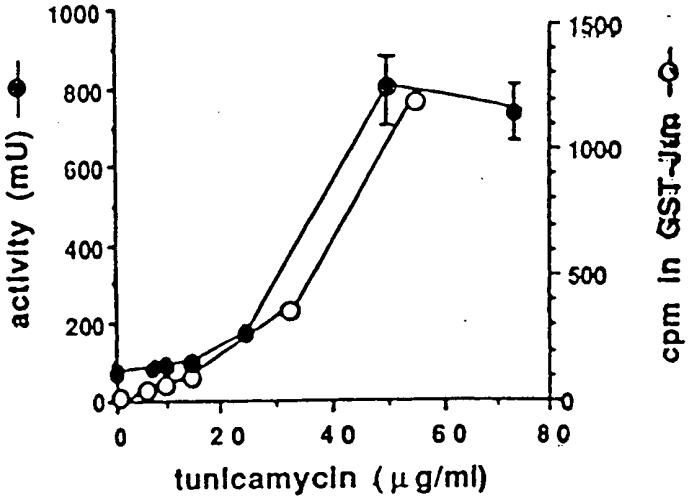

1000
800
600
400
200
0
activity (mU)
1500
1000
500
0
cpm in GST-Jun
0
20
40
60
80
tunicamycin (μg/ml)

FIG. 7B

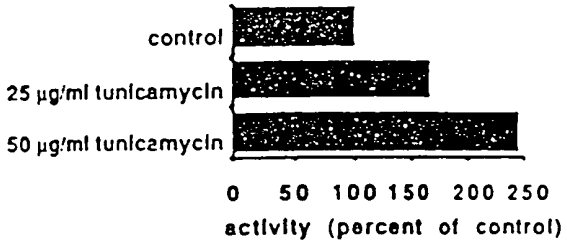

control
25 μg/ml tunicamycin
50 μg/ml tunicamycin
0
50
100
150
200
250
activity (percent of control)

FIG. 8A

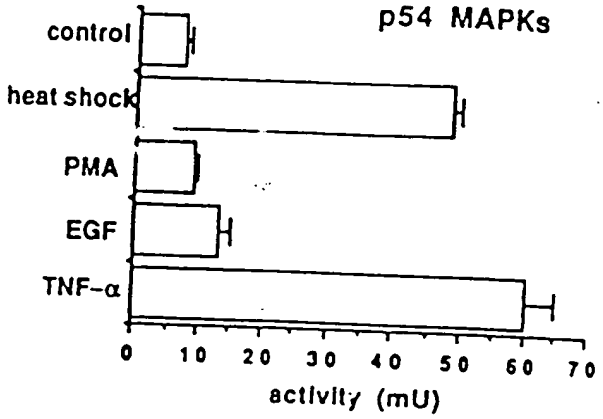

p54 MAPKs
control
heat shock
PMA
EGF
TNF-α
0
10
20
30
40
50
60
70
activity (mU)

FIG. 8B

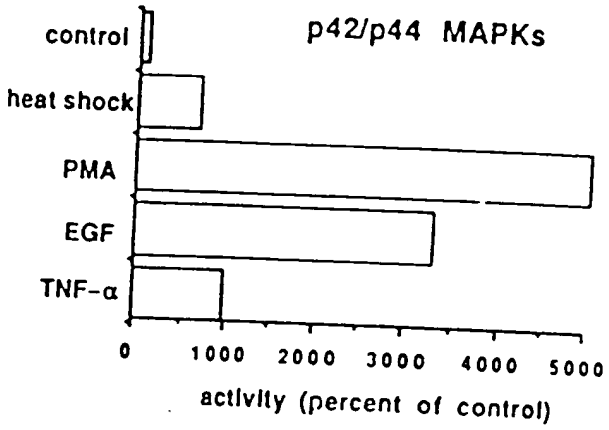

p42/p44 MAPKs
control
heat shock
PMA
EGF
TNF-α
0
1000
2000
3000
4000
5000
activity (percent of control)

FIG. 9A

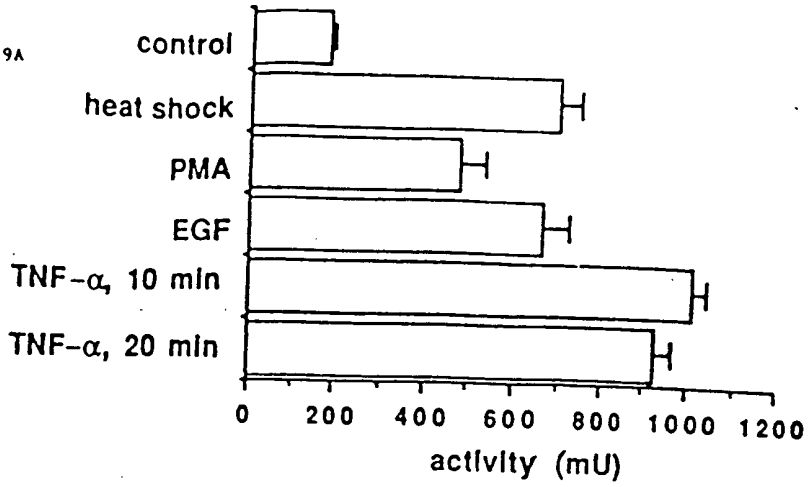
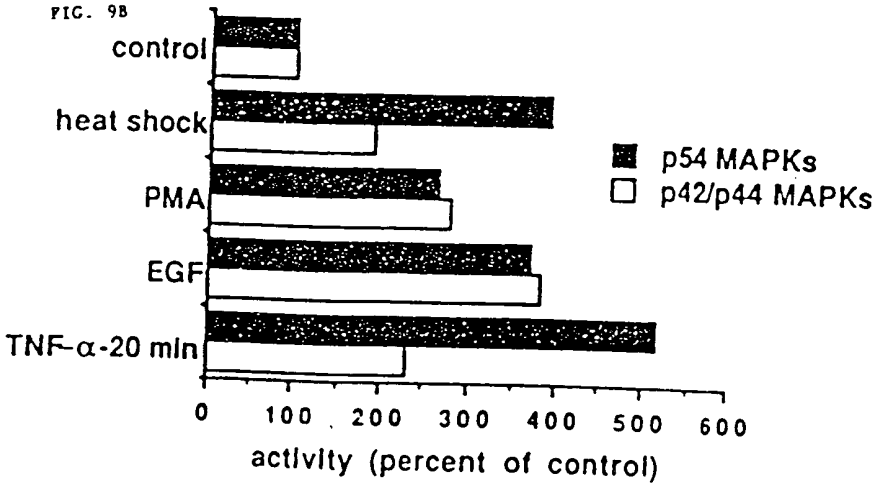

control
heat shock
PMA
EGF
TNF-α, 10 min
TNF-α, 20 min
0
200
400
600
800
1000
1200
activity (mU)

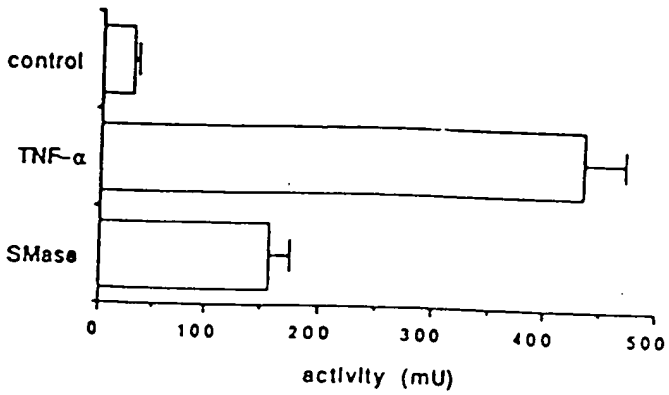
FIG. 9B


control
heat shock
PMA
EGF
TNF-α-20 min
p54 MAPKs
p42/p44 MAPKs
0
100
200
300
400
500
600
activity (percent of control)

11/14

2148898

FIG. 10



Effect of IL-1-β on EL-4 cell MAPKs

FIG. 11A

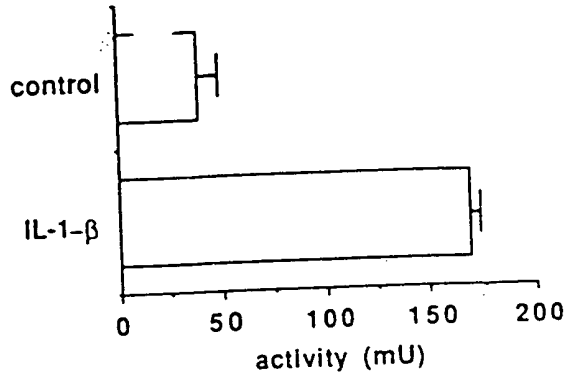


FIG. 11B

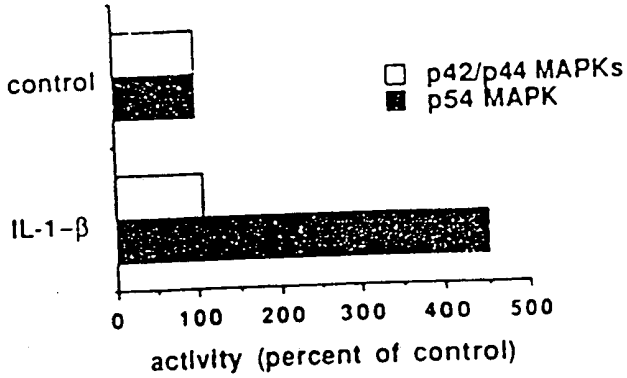


FIG. 12A

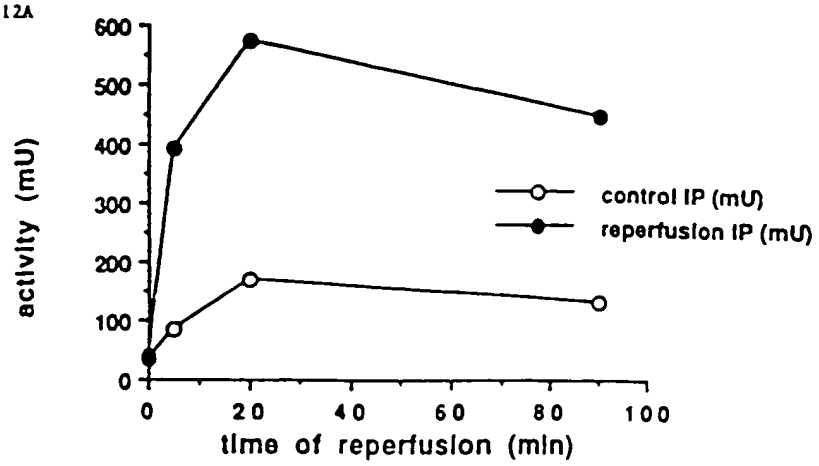


FIG. 12B

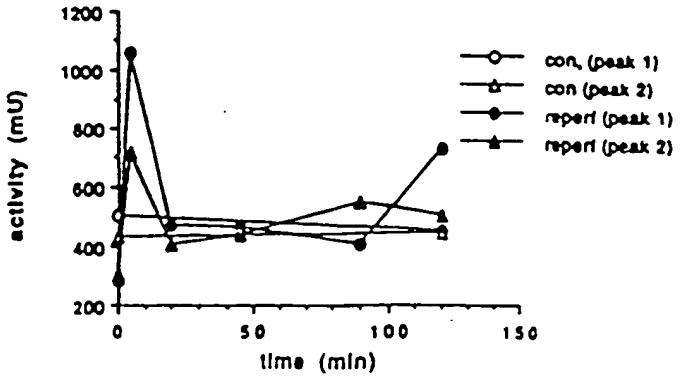


FIG. 13 2148898

Activation of rat GST-SAPK-α by extracts of TNF-treated HepG2 cells